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PHYSIOLOGICAL AND CLINICAL CHEMISTRY

BARTLEY

MANUAL OF

PHYSIOLOGICAL AND CLINICAL

CHEMISTRY

BY

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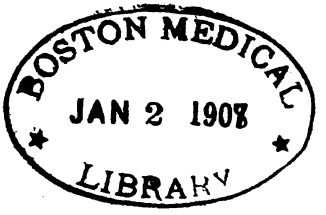

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PREFACE TO THE SECOND EDITION.

That there is considerable difference of opinion among teachers of chemistry as to what portions of the science can, with most profit, be presented to the medical student, is evidenced by the number and variety of small laboratory manuals for the use of medical students that have appeared within the past few years.

Some of these books deal largely with the detection of poisons, a subject that in actual practice is always entrusted to the professional chemist.

Others devote a great deal of attention to training the student in analytical methods, to sanitary analysis, or to the examination of foods or drugs. Another class devote most attention to the chemistry of the tissues and secretions and proximate principles of the human body. All of them, however, recognize the importance of the gross analysis of urine, many however, treating the subject from the purely chemical side.

While it will be admitted that all these subjects are important for medical students to know, it must also be admitted that the time usually given to the subject of chemistry is too limited for such an extensive course.

A medical college is a technical school for the training of young men or women in the science of the prevention or the diagnosis and treatment of disease.

The chemical teaching should, therefore, be directed to this purpose, and should consist in teaching the fundamental principles and the application of these principles to the science of medicine, especially to the diagnosis and treatment of diseased conditions. Such applications of the science of chemistry can rightfully be grouped under the head of *clinical chemistry*.

This little manual, the second edition of which is now presented, is the outcome of this idea gradually developed during the past twenty years of teaching in the Long Island College Hospital.

In preparing this edition the author has thoroughly revised the book, added much new matter including many new processes, some of which are original. It has been the aim of the author to give the

processes in sufficient detail to serve as working directions and yet to make them as concise as possible. Explanatory notes and a brief statement of the clinical significance of results obtained in the laboratory accompany the description of methods of procedure.

No attempt has been made to give a complete guide to the microscopic study of blood or to enter the field of general microscopic diagnosis.

While such a manual must of necessity be largely a compilation, many of the processes are either original or modified by the author. How well the aim of the author has been carried out others must determine. Credit has been given to the authors of special processes where possible. The author would here express his indebtedness to Dr. F. Blumenthal for several illustrations of urinary deposits from his work on "Die Pathologie des Harnes," and to the Publishers for the loan of illustrations from Landois' Physiology.

It is believed that this manual will be found to contain all that the physician will need as a guide to the ordinary clinical examination of urine, gastric contents, blood, feces and milk.

E. H. BARTLEY.

BROOKLYN, June, 1904.

CLINICAL CHEMISTRY.

EXERCISES IN EXPERIMENTAL PHYSIOLOGICAL CHEMISTRY.

The student should refer to the descriptive part of the text-book for the subjects treated of here.

THE SACCHARIDS OR CARBOHYDRATES.

1. Note the general appearance of the specimens of cane-sugar, milk-sugar, grape-sugar or dextrose, dextrin, and starch which are passed around.

2. *Solubility.*—Put some of each into cold water. Starch is insoluble; dextrose, lactose, and dextrin slowly dissolve, but more readily in hot water.

3. *Furfural Test. Molisch's Test.*—To 4 c.c. of a weak solution of any one of the above sugars, add three drops of a 15 per cent. alcoholic solution of alpha-naphthol, and then pour this carefully down the side of a test-tube containing about 2 c.c. strong H_2SO_4. There is formed a violet ring at the line of contact of the two liquids, which becomes more intense on gentle agitation.

This reaction is a general one for all the soluble carbohydrates.

THE PENTOSES, $C_6H_{10}O_5$.

1. Dip a pine splinter in some strong HCl, and then moisten with anilin acetate. The pentosane in the wood is converted into pentose by the HCl, and this into furfural, which gives a bright red color with anilin acetate, or with a solution of phloroglucin.

2. To about 2 grms. of crushed malted barley in a test-tube, add 2 c.c. HCl, dilute with 5 c.c. of water and boil. Hold over the mouth of the test-tube a piece of anilin acetate paper. The paper is reddened if pentoses are present.

THE HEXOSES, $C_6H_{12}O_6$.

These tests may be performed with a 2 per cent. solution of commercial glucose, or with a solution of cane-sugar of the same strength, which has been inverted by heating on a water-bath for 15 minutes with 4 or 5 drops of H_2SO_4 to 50 c.c. of the solution.

1. *Moore's Test.*—To 4 c.c. of the solution in a test-tube add 2 c.c. of a strong NaOH solution and boil. The liquid changes color, first becoming yellow and then brown. The depth of color increases with the quantity of sugar present.

2. *Fehling's Test.*—Boil 4 c.c. of Fehling's solution diluted with twice its volume of water and add 1 c.c. of the solution. Red cuprous oxide, Cu_2O, is precipitated.

3. To 10 c.c. of the solution add 5 c.c. of Barfoed's copper solution, and boil for three to five minutes. A red precipitate forms with the hexoses, but not with cane-sugar, lactose, maltose or dextrin.

Barfoed's Solution is prepared by dissolving 7 grms. of copper acetate in 100 c.c. of water and adding 2.5 c.c. of 38 per cent. acetic acid.

4. Rub up some of the solution with a small lump of yeast. Fill a test-tube with this mixture, close the tube with a perforated cork stopper, excluding all air, invert the tube in a vessel containing a little water, and let set from 12 to 24 hours, in a warm place. Fermentation takes place generating CO_2, which collects in the upper end of the tube.

$$C_6H_{12}O_6 + \text{yeast} = 2C_2H_5OH + 2CO_2.$$

5. To 15 c.c. of the solution in a wide test-tube add about 0.5 grms. of phenyl-hydrazin hydrochloride, and 1 grm. sodium acetate. Set the tube in boiling water for half an hour and cool. Yellow crystals separate on cooling, which under the microscope appear as rosettes or bundles of fine needles. If the precipitate appears amorphous, under the microscope, filter, dissolve the residue on the paper in hot alcohol, dilute with water, boil off the alcohol and cool, when the characteristic yellow stellate crystals will be formed. Williamson applies this test as follows:

Fill a test-tube to a depth of one half inch, with phenyl-hydrazine hydrochloride, then to the same depth with sodium acetate, then half full, with the liquid to be tested. Boil the liquid for two or three minutes, and set aside to cool, when the crystals of phenyl-glucosazone will be found in the bottom of the tube. The reaction is:

$$C_6H_{12}O_6 + 2C_6H_5N_2H_3 = C_{18}H_{22}N_4O_4 + 2H_2O + H_2.$$

The crystals are nearly insoluble in water even at 100° C., while the pentosazones dissolve at 60° C. Glucosazone and levulosazone, which seem to be identical, melt at 204° to 205° C., while pentosazones melt at 157° to 160° C.

DISACCHARIDS: CANE-SUGAR, MILK-SUGAR, MALTOSE, $C_{12}H_{22}O_{11}$.

Perform the above tests with these sugars and note the difference in behavior.

1. To a small quantity of each of the sugars on a watch glass, or in a test-tube, add a small quantity of strong H_2SO_4 and stir with a glass rod. Cane-sugar blackens, while maltose becomes slightly reddish or brown, but not black. Lactose remains unchanged.

2. Test the solubility of the three sugars in absolute alcohol. Maltose is soluble. Lactose and saccharose are insoluble.

POLYSACCHARIDS: DEXTRINS, STARCHES, CELLULOSES, ETC.

Dextrins are soluble in water. Starch and celluloses are insoluble.

1. To a solution of commercial dextrin, add a few drops of aqueous solution of iodine. A reddish-brown color shows the presence of *erythrodextrins*. *Achroodextrin* gives no color with iodine.

2. To some of the solution of dextrin add one half its volume of a one per cent. solution of tannin. *Dextrin* gives no precipitate; distinction from *soluble starch*, *gelatin* and *albumin*.

3. To 10 c.c. of alcohol in a test-tube add 2 c.c. of the concentrated dextrin solution. The dextrin is precipitated.

4. To a test-tube half full of a 1 per cent. solution of starch, diluted with three or four times its volume of water and warmed to about 40° C., add 1 c.c. of a solution of diastase, or 3 c.c. of saliva, and draw out a drop from time to time, and test on a cold white surface with a drop of aqueous iodine solution. (See (3) under Starch.)

5. *Starch.* — Examine microscopically the scrapings from the surface of a freshly cut potato. Note the appearance of the starch-grains, with their concentric markings.

On boiling starch with water, an opalescent solution is formed, which, if concentrated, gelatinizes on cooling.

Prepare about a 1 per cent. solution of starch for use in the following tests:

1. Add to a portion of the cold solution a few drops of aqueous iodine solution. An intense blue color is produced, which disappears on heating, and, if not heated too long, reappears on cooling. Prolonged heating drives off the iodine, and consequently no blue color returns on cooling.

2. Conversion into dextrin and dextrose. To some starch solution in a flask add a few drops of 25 per cent. sulphuric acid and boil for fifteen minutes. Take out a portion of the liquid at intervals of five minutes and test for dextrin and dextrose.

3. Prepare some *malt extract* by digesting about 10 gm. of powdered malt with 50 c.c. of water at 40° C. for fifteen minutes and filter through absorbent cotton. This extract contains the diastasic or malting ferment.

4. To 10 c.c. of the starch solution add 2 c.c. of the malt extract, and place the mixture in a water-bath at 40° C. At intervals of one minute test portions of the liquid by mixing a drop of it with a drop of aqueous iodine solution on a testing slab. The blue color at first seen is soon replaced by a violet (mixture of blue and red), and then by a reddish-brown (due to *erythrodextrin*), which gradually vanishes. Alcohol added to the liquid when all starch and erythrodextrin have gone, still causes a precipitate of a dextrin, which, as it gives no color with iodine, is called *achroodextrin.* The liquid also contains a reducing sugar — maltose.

5. Repeat test No. 4 with saliva instead of extract of malt.

Glycogen dissolves in water, forming an opalescent solution, like soluble starch.

1. To a small portion of a 1 per cent. solution of glycogen, add a few drops of iodine solution. A reddish-brown color is produced, which disappears on heating and reappears on cooling. Resemblance to erythrodextrin and soluble starch. Peptone interferes with this test.

2. Heat 5 c.c. of Fehling's solution to boiling, and add 1 c.c. of the solution of glycogen. No reduction.

3. To 5 c.c. of the solution, warmed to 40° C. add 1 c.c. of saliva, and test the solution, from time to time, with iodine solution on a white surface. After ten minutes, test with Fehling's solution, as in 2, under hexoses.

4. To another portion of the solution add 2 or 3 drops of HCl, and boil a few minutes. Neutralize with NaOH, and heat with Fehling's solution. It gives reaction for *dextrose.*

Cellulose, is insoluble in water, alcohol, dilute acids or alkalies.

1. It is soluble in strong H_2SO_4, which solution gives a blue color with iodine owing to formation of an *amyloid substance.* When the above solution is diluted at once, the amyloid substance precipitates, but on standing some hours, or after heating, no precipitates forms and the solution reduces alkaline copper solutions, showing the presence of dextrose.

2. Warm some shreds of filter paper or absorbent cotton, with Schweizer's reagent. The cellulose dissolves. It is precipitated as a white amorphous solid on acidifying the solution with acetic acid.

Schweizer's reagent is made by adding to a solution of $CuSO_4$ some NH_4Cl and then NaOH in slight excess.

Filter off the precipitated CuO_2H_2, wash, and dissolve in strong ammonia water (20 per cent.).

3. Into a cold mixture of two volumes of H_2SO_4 and one of water, dip a piece of dry filter paper for a few seconds, remove and wash at once in water. Note the production of parchment paper. (See 1 above.)

A systematic scheme for the identification of the most important carbohydrates.

STEP 1.

General reaction.

To 5 c.c. of a weak solution of the substance add a few drops of 15 per cent. alcoholic solution of alpha-naphthol, and overlay this on strong sulphuric acid; a violet or blue zone at the line of contact indicates *a carbohydrate.*

If the substance is insoluble in water, dissolve in 25 per cent. sulphuric acid and float over this solution a mixture of water and alpha-naphthol, and observe as above.

STEP 2.

Shake about 1 gm. of the substance with 10 c.c. of water in a test-tube. Decant or filter from any insoluble residue, which may be starch or cellulose. Save the filtrate for the succeeding steps, and test the residue as follows:

To the insoluble matter in the test-tube (not on the filter paper) add a few drops of a dilute aqueous solution of iodine; a blue color indicates *starch.*

STEP 3.

Take a portion of the filtrate obtained in step 2 and divide into two parts.

To one part add an equal volume of strong alcohol; a precipitate indicates *dextrin.*

To the other part on a white slab add a few drops of dilute solution of iodine; a blue color indicates *cooked starch,* and a reddish-brown color, *dextrin.*

THE QUALITATIVE DETECTION OF THE COMMON SUGARS. (*Allen.*)

	Dextrose, $C_6H_{12}O_6$.	Levulose, $C_6H_{12}O_6$.	Milk-sugar, $C_{12}H_{22}O_{11} + H_2O$.	Maltose. $C_{12}H_{22}O_{11} + H_2O$.	Cane-sugar, $C_{12}H_{22}O_{11}$.	Dextrin. $(C_6H_{10}O_5)_n$.
1. Moisten the solid sugar with water, and stir in the cold with concentrated sulphuric acid (1.845 sp. gr.).	Not affected when pure.	Not affected when pure.	Not affected.	Slightly reddish or brownish, gradually turning darker.	Charred.	Not affected.
2. Triturate the solid sugar with caustic soda, or boil it with a 3 per cent. caustic soda solution for one minute.	Deep-brown coloration.	Deep-brown coloration.	Not affected.	Slightly discolored.	Not affected.	Not affected.
3. To the neutral aqueous solution add a few drops of Fehling's copper solution, and heat to boiling for a few minutes.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Not affected.	Not affected.
4. Heat the solution to boiling for half an hour with one-twentieth of its bulk of strong sulphuric acid, neutralize with soda, and heat to boiling with Fehling's solution for a few minutes.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	Red precipitate of Cu_2O
5. To a few drops of Fehling's solution add caustic soda and ammonia, heat to boiling, and add the saccharine solution drop by drop, keeping the liquid boiling (Pavy's test).	Liquid decolorized.	Liquid decolorized.	Liquid decolorized.	Liquid decolorized.	No change.	No change.
6. Boil the solution for two minutes with 1 c.c. of a liquid containing 4 per cent. of cupric acetate, and 1 per cent. of acetic acid ($C_2H_3O_2H$).	Red precipitate of Cu_2O.	Red precipitate of Cu_2O.	No change.	No change.	No change.	No change.
7. Observe the solution in the polariscope.	Dextro-rotatory.	Levo-rotatory.	Dextro-rotatory.	Dextro-rotatory.	Dextro-rotatory.	Dextro-rotatory.
8. Heat the solution with diluted acid, as in test 4, and observe again in the polariscope.	Dextro-rotatory power unchanged.	Levo-rotatory power unchanged.	Dextro-rotatory power increased.	Dextro-rotatory power diminished.	Dextro-rotatory power changed to levo-rotatory.	Dextro-rotatory power diminished.

STEP 4.

Boil a portion of the filtrate obtained in step 2 with Barfoed's solution of copper acetate; a precipitate of cuprous oxide indicates either *dextrose* or *levulose*, or both.

If a precipitate forms, filter, and use the filtrate, or solution in which Barfoed's reagent produced no effect, as follows:

STEP 5.

To this filtrate or solution add an excess of solution of basic lead acetate, filter, and to the filtrate add an excess of solution of sodium sulphate to precipitate lead, and then filter.

To the filtrate which must still be blue (if not, add a few drops of copper sulphate solution) add sodium hydroxide to make alkaline, and heat to boiling; a red precipitate of cuprous oxide indicates *maltose* or *lactose*, or both.

Filter and save the filtrate (or the solution if no effect was produced) for step 6.

To determine whether maltose or lactose or both are present, proceed as follows: Treat a small quantity of the filtrate obtained in step 2 with ammonium hydroxide in excess, add a few drops of alkaline bismuth solution, and set the tube in water at a temperature of about 60° C.; *maltose* solutions reduce the bismuth while lactose does not at this temperature within half an hour.

To detect lactose proceed as follows: To about 5 c.c. of strong nitric acid in a test-tube add about 0.5 gm. of the original substance, and warm gently until red fumes begin to come off. Set the tube in about 200 c.c. of hot water, and allow it to remain there until cold; in a few hours a white crystalline mucic acid separates if *lactose* is present.

STEP 6.

To the filtrate obtained in step 5 add a few drops of sulphuric acid and boil for a few minutes, neutralize with excess of sodium hydroxide, add a few drops of copper-sulphate solution, and heat to boiling; a red precipitate of cuprous oxide indicates *saccharose* (cane sugar).

FATS.

Tests to be practised upon pure lard.

1. Note that it is insoluble in water. Test its solubility in alcohol, ether, chloroform, and petroleum-ether.

2. Boil a small portion of the fat with an equal volume of alcohol and NaOH solution. It dissolves and yields a solution of soap.

3. Add to this solution a few drops of 25 per cent. sulphuric acid. On heating and diluting with water a layer of fatty acid collects on the surface.

4. To a small portion of the solution obtained in 2, add some water and then solution of $CaCl_2$. The precipitate is an insoluble calcium soap. Add a few drops of this same diluted solution, to some tap-water in a beaker, and explain the result.

5. To another portion of the solution obtained in 2, add a solution of $Pb.(C_2H_3O_2)_2$. A white sticky precipitate of lead soap forms.

6. To about 1 c.c. of cod-liver oil, add 5 c.c. of alcohol and boil for some minutes, and test the reaction of the solution with litmus paper. Try the same experiment with a rancid oil.

7. To 5 c.c. of each of the two oils used in 6, add 25 c.c. of a 1 per cent. solution of Na_2CO_3, shake into emulsions and set away for observation as to the permanency of the emulsions. The rancid oil emulsifies the more readily, and forms the more permanent emulsion.

8. Prepare two test-tubes, the one half full of a strong aqueous solution of common hard soap, and the other with pure water. Now add to both about the same amount of lard, and shake vigorously. Note the difference in behavior, especially on standing a few min-ntes.

9. Rub up a small lump of lard with an equal quantity of $KHSO_4$, transfer to a dry test-tube, and heat cautiously. The irritating, pungent, odor of *acrolein* or *acrylic-aldehyde* is obtained from the dehydration of the glycerol.

$$C_3H_5(OH)_3 = C_2H_3COH + 2H_2O.$$

10. Repeat this experiment with glycerol and with a piece of stearin candle in place of the lard. Stearin, used in making candles, is a mixture of stearic and palmitic acids, and contains no glycerol.

THE PROTEIDS.

Tests for Proteids. — The following tests are to be tried with a mixture of one part of white of egg to ten of water. (Egg-white contains a mixture of albumin and globulin.)

1. **Heat Coagulation.** — Boil a portion of the solution. It does not coagulate. Faintly acidulate the hot solution with a few drops of 2 per cent. acetic acid. The proteid is rendered insoluble (coagulated proteid) in presence of a slight excess of acid.

2. **Precipitation with Nitric Acid.** — The addition of strong nitric acid to the original solution also produces a white precipitate.

3. **Xanthoproteic Reaction.** — On boiling, the white precipitate produced by nitric acid turns yellow; after cooling, add ammonia; the yellow color changes to orange.

4. **Millon's Test.** — Millon's reagent, which is a freshly prepared solution of the nitrate of mercury containing excess of nitric acid, gives a white precipitate, which turns brick-red on boiling.

5. **Bromine water** precipitates all forms of proteids from solutions acidified with HCl.

6. After the addition of acetic acid, potassium ferrocyanide gives a white precipitate.

7. Add a drop of a 1 per cent. solution of cupric sulphate to the original solution and then caustic potash; a violet solution is obtained only after boiling.

8. Repeat experiment 7 with a solution of commercial peptone, and note that a rose-red (biuret) color is obtained.

9. **Adamkiewicz's Reaction.** — To 2 c.c. of H_2SO_4 add 4 c.c. of glacial acetic acid. To this mixture, add a few drops of the albumin solution, and warm gently. A reddish violet color is given by albumin, but not by gelatin or gelatin peptone.

10. **Action of Neutral Salts.** — The following experiments may be performed with blood serum, which like egg-white, contains albumin and globulin:

(*a*) Saturate it with magnesium sulphate, by adding crystals of the salt to the serum and shaking vigorously in a flask. A white precipitate of serum globulin is produced. Filter. The filtrate contains serum albumin. Saturate the filtrate with $(NH_4)_2SO_4$ or $ZnSO_4$, which precipitates the albumin. (Serum-globulin is incompletely precipitated by diluting the serum with a large quantity of water, and also by passing a stream of carbonic acid gas through diluted serum.)

(*b*) Saturate another portion of the serum with ammonium sulphate or zinc sulphate; a precipitate is produced of both the globulin and albumin. Filter. The filtrate contains no proteid.

11. Repeat the last experiment with a solution of commercial peptone. A precipitate is produced of the albumoses or proteoses it contains. Filter. The filtrate contains the true peptone. Ammonium sulphate and zinc sulphate precipitate all proteids except peptones.

12. **Action of Acids and Alkalies on Albumin.** — Take three test-tubes and label them A, B, and C.

In each place 10 c.c. of diluted egg-white, similar to that used above.

To A add 1 c.c. of 0.1 per cent. solution of NaOH.

To B add 1 c.c. of 0.1 per cent. solution of NaOH.

To C add a rather larger amount of 0.1 per cent. sulphuric acid.

Put all three into the warm bath at about the temperature of the body (36°–40° C.).

After ten minutes remove test-tube A and boil. The proteid is no longer coagulated by heat, having been converted into **alkali-albumin.** After cooling, color with phenol-phthaleïn solution and neutralize with 0.1 per cent. acid. At the neutral point a precipitate is formed which is soluble in excess of either acid or alkali.

Next, remove B. This also now contains alkali-albumin. Add to it a few drops of sodium phosphate, color with phenol-phthaleïn, and neutralize as before. Note that the alkali-albumin now requires more acid for its precipitation than in A, the acid first added converting the sodium phosphate into acid sodium phosphate.

Now remove C from the bath. Boil it. Again there is no coagulation, the proteid having been converted into **acid-albumin.** After cooling, add three drops of phenol-phthaleïn and neutralize with 0.1 per cent. NaOH. At the neutral point a precipitate is formed, soluble in excess of acid or alkali. As acid-albumin is formed more slowly than alkali-albumin, it is best to leave this experiment until the last.

PROTEOSES OR ALBUMOSES AND PEPTONES.

Use a solution of somatose, or of Mosquera beef-meal.

Make a solution of one of these substances in 10 per cent. sodium chloride solution, and filter. Very little residue may be left on the filter. This may consist of dysalbumose, an insoluble form of hetero-albumose, formed during the process of preparing the substance, or of unchanged proteid. If hot saline solution is used as the solvent, instead of cold, this amount of insoluble residue is increased, hetero-albumose being to a slight extent precipitated by heat.

The solution gives the following tests:

1. It does not coagulate on heating.

It gives the general proteid reactions.

2. Biuret reaction (due both to peptone and albumoses). (See 8, under Proteids.)

3. A drop of nitric acid gives a precipitate, with the primary albumoses present, which dissolves upon warming and reappears on cooling.

4. For the separation of the albumoses and peptone proceed as follows:

(*a*) Saturate the solution with ammonium sulphate or zinc sulphate and filter. The filtrate contains the peptone and the precipitate the albumoses. The peptone is not precipitated by nitric acid, nor by most of the reagents that precipitate other proteids. It is precipitated completely by alcohol, tannin, and potassio-mercuric iodide; imperfectly by phospho-tungstic and phospho-molybdic acids. It gives the biuret

reaction, but in the presence of ammonium sulphate a large excess of caustic potash is necessary.

(*b*) Dialyze another portion of the solution; hetero-albumose is precipitated.

(*c*) Saturate another portion of the solution with sodium chloride after faintly acidulating with acetic acid. Proto-albumose and hetero-albumose are precipitated. Filter. The filtrate contains the deutero-albumose and peptone.

The proto- and hetero-albumose may be redissolved by adding distilled water, and may be separated from each other by dialysis.

Deutero-albumose may be separated from the peptone by saturating the solution with ammonium sulphate or zinc sulphate, or by the addition of a crystal of glacial phosphoric acid. These reagents precipitate the deutero-albumose, but not the peptone.

Deutero-albumose gives the nitric-acid reaction (see 3) characteristic of the albumoses only in the presence of excess of salt. If the salt is removed by dialysis, nitric acid then causes no precipitate.

GELATIN.

Make a 2 per cent. solution, of a clear transparent gelatin, with warm water.

1. Boil a small portion of this solution. It does not coagulate, even after acidifying.

2. Saturate 10 c.c. of the cold solution, with $MgSO_4$ and another portion with Na_2SO_4. Both precipitate gelatin.

3. Perform experiments 2, 3, 5, 6 and 7 described under proteids.

4. Make a strong solution of gelatin in warm water and let set until cold. It sets into a jelly.

SALIVA.

Collect some saliva by chewing a small quantity of paraffin and expectorating in a suitable vessel.

1. To a little saliva in a test-tube add acetic acid. Mucin is precipitated in stringy flakes.

2. Filter some fresh saliva, to separate cells and mucus, and apply the xanthoproteic or Millon's test to the filtrate; the presence of proteid is shown.

3. Put some 0.5 per cent. starch solution into four test-tubes. Add some filtered saliva to A. To B add saliva and three drops of HCl; to C add 1 c.c. of NaOH solution; to D add saliva, and boil. Put all the tubes in the water-bath at 40° C. After ten minutes, remove and test them with iodine, and Trommer's test. The saliva in A will be found to have converted the starch into dextrin and maltose. In B and C no sugar is formed, because strong acids and alkalies arrest the action of saliva on starch; nor in D, because the diastase was destroyed by heat.

4. The presence of potassium sulphocyanide (KCNS) in saliva may be shown by the red color given by a drop of ferric chloride. This color is discharged by mercuric chloride.

5. The reaction of saliva is usually alkaline to litmus paper.

6. Examine some saliva with the microscope, using a 1/6 in. objective. Note the epithelial cells from the mouth, and the granular salivary corpuscles, larger than the leucocytes.

PEPTIC DIGESTION OF PROTEIDS.

1. Half-fill four test-tubes —

A with water.

B with 0.2 per cent. hydrochloric acid.

C with 0.2 per cent. hydrochloric acid.

D with solution of white of egg (1 to 9 of water).

2. To A add 1 c.c. of a 2 per cent. solution of a good pepsin, and a piece of a solid proteid, as fibrin or coagulated egg-white.

To B also add 1 c.c. of the pepsin solution and a piece of fibrin or coagulated egg-white.

To C add only a piece of fibrin, or coagulated white of egg.

To D add 1 c.c. of pepsin solution and fill up the tube with 0.4 per cent. hydrochloric acid.

3. Put all four tubes into the water-bath at 40°C., and observe them from time to time.

In A the albumin (or fibrin) remains unaltered.

In B it becomes swollen, and gradually dissolves.

In C it becomes swollen, but does not dissolve.

4. Examine the *solution* in test-tube B.

(*a*) To 5 c.c. of the liquid add two drops of phenol-phthaleïn and neutralize with dilute alkali. Acid-albumin or syntonin is precipitated.

(*b*) Take another test-tube and put into it a drop of solution of copper sulphate; empty it out so that the merest trace of copper sulphate remains adherent to the wall of the tube; then add a portion of the solution from test-tube B and a few drops of NaOH or KOH solution. A pink color (biuret reaction) is produced. This should be carefully compared with the violet tint given by unaltered albumin.

(*c*) To a third portion of the fluid, in test-tube B, add a drop of nitric acid; primary albumoses or propeptones are precipitated. This precipitate dissolves on heating and reappears on cooling.

5. These three tests should be repeated with the digested white of egg in test-tube D, after boiling and filtering, to remove any unchanged albumin.

PANCREATIC DIGESTION.

1. A 1 per cent. solution of sodium carbonate, to which a little glycerine extract of pancreas has been added, forms a good artificial

pancreatic fluid. In place of this we may use one of the commercial solutions of pancreatic extract, diluted with an equal volume of 1 per cent. Na_2CO_3.

2. Half fill three test-tubes with one of these solutions:

A. To this add half its volume of diluted egg-white (1 in 10),

B. To this add a piece of fibrin.

C. Boil this, cool, then add fibrin.

Put all into the water-bath at 40° C. After half an hour, test A and B for alkali-albumin by neutralization with dilute acetic acid, and filter. Boil the filtrate to precipitate any unchanged albumin, and filter again. Test the filtrate for albumoses by 2 and 3, p. 22, and for albumoses and peptone by the biuret reaction as under peptic digestion.

Note that the fibrin in B does not swell up and dissolve as in peptic digestion, but that it is eaten away from the edges to the interior.

In C no digestion occurs, as the ferments have been destroyed by boiling.

3. Add equal quantities of a solution of starch to three test-tubes:

A. To this add a few drops of pancreatic extract.

B. To this add a few drops of bile.

C. To this add both bile and pancreatic extract.

Put these into the water-bath and test small portions of each every half minute by the iodine reaction. The blue color-reaction disappears first in C, then in A while B undergoes no change. Now test A and C for maltose by Trommer's test.

4. Shake up a few drops of olive oil with the above pancreatic extract. A milky fluid (emulsion) is formed, from which the oil does not readily separate on standing. Repeat this experiment with the addition of one tenth volume of ox-bile.

The foregoing experiments illustrate the action of pancreatic juice on all three classes of foods.

THE EXAMINATION OF ARTIFICIAL FOODS.

The following scheme will serve as a general guide in the examination of prepared foods for the detection of the proximate principles. For confirmatory tests the student should consult the preceding exercises:

1. If the food be a liquid, dilute and apply the tests at once. If a powder or paste, proceed as follows: Shake a portion of the food in a test-tube with ether, evaporate the ether, and examine the residue for fat or oil globules. If fat is found to be present, shake a portion of the food repeatedly with ether, pouring off the ether each time before

adding fresh ether, until the fat is all removed. Warm the undissolved residue, to drive off the ether, and shake about 5 gm., if a powder or paste, with 50 c.c. of cold water and filter. Wash the residue thoroughly, with cold water, and reserve for treatment under 2. Label the filtrate *solution A*.

Note the color, odor, transparency or opalescence, taste, smell, solubility, reaction, etc. *This solution can not contain uncooked starch, fibrin, gelatin, or fats. If neutral in reaction, albuminates are absent, and need not be tested for.*

Dark-colored liquids suggest blood, meat extract, malt, etc.

Light-colored liquids suggest milk solids, certain proteids, or carbohydrates.

Opalescence suggests cooked starch in solution, glycogen, or globulins, caseinogen or fat emulsion.

2. Boil a portion of the residue insoluble in cold water, with water; filter while hot and examine the filtrate, when cold. *This solution will contain any starch or gelatin present in the food. It will not contain albumin or globulin, and albuminates only in acid or alkaline solutions.* Mark this *solution* B. Wash the residue well by decantation, or on the filter, with hot water, and reserve for examination under No. 11, below. Examine the cold and hot solutions A and B, separately, as follows:

3. Add iodine to small portions of the filtrate.

Blue color indicates starch. Confirm by conversion into sugar with saliva or dilute H_2SO_4. (See 2 and 4 under Starch.)

Reddish-brown color indicates dextrin or glycogen. Glycogen forms opalescent solutions. Both glycogen and dextrin are precipitated by strong alcohol and basic lead acetate. Both form reducing sugars when boiled with dilute H_2SO_4.

4. Add copper sulphate and caustic soda to the aqueous solution.

(*a*) Blue solution: Boil; yellow or red precipitate. **Dextrose, maltose,** or **lactose.** Found only in solution A. (For distinguishing tests see under Carbohydrates.)

(*b*) Blue solution: A very slight reduction on boiling.

Boil some of the solution A with dilute sulphuric acid, and then boil with copper sulphate and caustic potash; abundant yellow or red precipitate: **Cane-sugar.** Found only in solution A.

(*c*) Violet solution: **Proteids** (albumins, globulins, albuminates) and **gelatin.**

(*d*) Pink solution: Biuret reaction. **Peptones** or **albumoses (proteoses).** Only a trace of copper sulphate must be used. (See 7 and 8 under Proteids.)

5. When proteids are present, proceed as follows: Test the reaction with litmus paper, and neutralize:

(*a*) Neutralization causes a precipitate soluble in excess of weak acid or alkali; **acid-albumin** or **alkali-albumin,** according as the reaction of the original liquid is acid or alkaline respectively. If the original liquid is neutral, acid-albumin and alkali-albumin must both be absent. A precipitate produced by acid, insoluble in excess, is probably caseinogen (see 6). Filter if necessary.

6. Acidify the filtrate obtained in 5 with a few drops of acetic acid and *warm gently* but *do not heat too hot.* Caseinogen precipitates, if present. Filter and boil the filtrate.

(*a*) Precipitate produced: **Albumins** or **globulins.**

(*b*) No precipitate: **Gelatin, albumose** or **peptones** may be present.

7. If albumin or globulin be present, remove according to 5.

(*a*) Saturate the filtrate with solid sodium sulphate or magnesium sulphate. Precipitate: **Gelatin** and **primary albumoses,** if present.

(*b*) Or, better, add one-fourth volume of solution of potassium dichromate. Precipitate: **Gelatin.**

8. Saturate the solution from 7 (*a*) from which albuminates, albumin, globulin, and gelatin have been removed, if present, with crystals of ammonium sulphate, or zinc sulphate.

(*a*) Precipitate: **Proteoses** or **albumoses.** (*b*) No precipitate: **Peptone** and **meat bases** may be present. If there is a precipitate, filter clear.

If both are present, the precipitate contains the proteoses, and the filtrate the peptone.

9. To a fresh portion of the solution from 7 (*a*), add a few drops of nitric acid.

(*a*) No precipitate, even though excess of sodium chloride be also added: No **albumose.**

(*b*) A precipitate only after adding sodium chloride: **Deutero-albumose.**

(*c*) Precipitate which disappears on heating and reappears on cooling: **Albumoses.** This is the distinctive test of all the proteoses or albumoses, and is given by all of them. For one of them, however (*deuteroalbumose*), excess of sodium chloride must also be added.

In all cases nitric acid *plus heat* causes a yellow color, turned orange by ammonia. (Xanthoproteic reaction.)

10. Remove all proteids from the solution by the addition of HCl and bromine water, or by slightly acidifying with acetic acid and adding a solution of tannin. Filter.

Test filtrate for **meat bases** and **amids:**

(*a*) Add excess of ammonia and then silver nitrate. A precipitate: Xanthin bases, creatinin, uric acid, etc.

(*b*) Acidify strongly with HCl and add phosphotungstic acid. The meat bases and amido-acids are precipitated. The precipitate dissolves on heating to 100° C., and reprecipitates on cooling.

11. The *residue insoluble in hot water*, should be examined under the microscope, for meat fiber, or vegetable cells, or parts of cells.

Test for cellulose, if suspected to be present with H_2SO_4 and iodine. Animal matters, as fiber, or fibrin are soluble in hot KOH or NaOH solutions, and are precipitated, on careful neutralization. Vegetable proteids behave like animal proteids, in above tests.

Interpretation of Results Obtained in the Examination of an Artificial Food.—Vegetable foods are characterized by starch, or by its derivatives, dextrin, maltose or dextrose. Sugar may be added to a food in nearly a pure state. Commercial glucose almost invariably contains dextrin.

Whether cooked or uncooked cereals are present, may usually be determined by the presence of soluble starch, dissolving in cold water and found in *solution A*. Dextrinized foods contain either no starch, or a small amount of starch, much dextrin, and some maltose. Predigested cereals show much reducing sugar (maltose) and some dextrin.

Milk foods or those containing milk solids form white, milky-looking solutions, which do not filter clear. Their solutions coagulate when acidified with acetic acid and warmed gently. The precipitate is easily soluble in dilute NaOH solution. They reduce Fehling's solution and give the mucic acid reaction with HNO_3. (See 2, Step 5, p. 19.)

Meat foods, or animal foods, are characterized by the large amount of *proteids* or *meat bases*. They are mostly of a dark color and have a rather unpleasant odor of meat extract, or gluey odor. In some the proteids coagulate on boiling after acidifying, in others the proteids are in the partially digested form and are precipitated by saturation with $(NH_4)_2SO_4$ or $ZnSO_4$. Few of them contain more than traces of peptones. The cooked meat preparations all contain more or less *gelatin*, precipitable by saturation of their solutions with the above salts and also by $MgSO_4$. Those made wholly from blood, casein or eggs, do not contain more than traces of the meat bases, while all others show a very considerable amount of these bases. Some of them contain meat fibers.

EXPERIMENTS WITH BEEF'S BLOOD.

1. Test the reaction with sensitive litmus paper previously wetted with a strong salt solution.

2. To about 5 c.c. of water add a few drops of blood and mix well. Now add a few c.c. of fresh tincture guaiac and about the same volume of old turpentine, agitate and let stand a few minutes. A deep indigo-blue color develops. *Pus* and sometimes *milk* give a similar color.

3. To a few drops of blood, add several times as much hydrogen dioxide. There is a strong effervescence, and evolution of oxygen. *Pus* gives the same reaction.

4. To 2 c.c. of fresh blood, add 10 c.c. of water, and 3 c.c. of acetic acid and agitate gently. The blood cells are disintegrated and dissolved, and the solution becomes clear — *laky blood.*

5. **Preparation of hemin crystals.** — Into a small Erlenmeyer flask add 10 c.c. of glacial acetic acid, heat on a water-bath and, when hot, add slowly 3 c.c. of defibrinated blood with constant agitation. Continue the heating for a half hour, transfer to a beaker or watch glass, and let set over night. Examine the sediment the next day, with a microscope, and sketch the dark brown crystals of *hemin.*

6. **Detection of Blood Stains.** — Place a small piece of blood-stained cloth on a microscopic slide, moisten with a few drops of 1 per cent. solution of NaCl, and squeeze out as much of the coloring matter as possible with the aid of a glass rod. Evaporate off the water, and moisten the residue with 2 drops of glacial acetic acid, cover with a cover-glass and heat again until most of the acetic acid has evaporated, cool and examine for the *hemin crystals*, with the microscope.

7. To about 10 c.c. of defibrinated beef's blood add about 90 c.c. water, acidulate very slightly with acetic acid, heat to boiling and filter. The coagulum of albumin and globulin is colored brown by hemoglobin. The filtrate is clear, or nearly so.

8. Evaporate the filtrate obtained in 7 to a small volume (about 20 c.c.) and filter again if a precipitate forms. Divide the filtrate into three portions.

(*a*) Boil some Fehling's solution, and add one portion to show presence of *dextrose.*

(*b*) Acidulate another portion with HNO_3 and add $AgNO_3$; precipitate shows the presence of *chlorides.*

(*c*) To another portion add NH_4OH and magnesia mixture, a white precipitate, soluble in dilute HCl, and reprecipitated on adding NH_4OH in excess indicates *phosphates.*

(*d*) To a few drops of blood, in a test-tube, add about the same number of drops of HNO_3, heat cautiously over the flame until the

blood is well decomposed, add a few drops of HCl and heat again, until a clear solution is obtained and only a few flakes of carbon remain undissolved. Dilute the mixture with 10 c.c. distilled water, and add a few drops of KCyS solution. A red color shows the presence of iron. By having a standard iron solution for comparison, this method may be made quantitative.

9. **Spectroscopic Examination.** — Add about 1 c.c. of blood to 50 c.c. of distilled water and shake thoroughly. Suspend a test-tube containing some of this diluted blood before the slit of a spectroscope, and between it and a luminous gas or lamp light. Observe the two dark bands — absorption bands — of *oxyhemoglobin* in the yellow. (See Table, p. 32, No. 1.) Place in the flame a platinum wire, previously wet with a strong salt solution. Note the position of the bands with reference to the yellow sodium line.

10. **Reduced Hemoglobin.** — To the tube before the spectroscope add a few drops of freshly prepared Stoke's solution, and observe immediately. The two bands gradually disappear, and there appears one broad band of *reduced hemoglobin*. (See Table of Spectra, p. 32, No. 2.) The color of the solution changes from blood red to a violet hue. *Stoke's solution* is prepared by dissolving a small crystal of $FeSO_4$ and about the same amount of tartaric acid, in some water and then adding to the solution NH_4OH to alkaline reaction. The reduction of the oxyhemoglobin to reduced hemoglobin may also be brought about by a strong solution of $(NH_4)_2S$. There is, however, often to be seen another smaller line in the red due to the band of *sulphohemoglobin*.

11. **Methemoglobin.** — To some diluted blood in a test-tube, add a few drops of a strong freshly prepared solution of potassium ferricyanide. The solution assumes a brown color, and shows an intense dark band, in the red, with two less dense bands to the right. (See Fig. 5.)

12. If now to the tube containing the methemoglobin, a few drops of solution of $(NH_4)_2S$ be added and agitated well with the air in the upper part of the tube, and again examined, the *oxyhemoglobin* bands appear, which gradually give place to the *reduced hemoglobin* band.

13. To some of the diluted blood add an equal volume of freshly prepared H_2S-water. The color changes to brown red and greenish, due to the formation of sulph-hemoglobin, which gives a spectrum resembling the methemoglobin, but it can not be reduced. (See Fig. 6.)

14. **CO-hemoglobin.** — Pass ordinary illuminating gas through some blood, or, put 25 c.c. of blood in a quart bottle and then displace the air with gas and let stand for a few hours with frequent shaking.

The color of the blood becomes cherry red. Dilute some of this blood and examine it with the spectroscope.

Two bands are seen, resembling those of oxyhemoglobin, but the bands are a little darker and a little to the right of those of oxyhemoglobin.

15. Add to some of the diluted carbon-monoxide blood a few drops of Stoke's solution, and examine the spectrum. Observe that it does not reduce. Try also experiment 11, above, and note the difference between this and ordinary blood.

16. Compare the color produced in the ordinary and CO-blood on the addition of half its volume of NaOH solution; also compare the behavior of the two bloods, when treated, as follows:

(*a*) To 10 c.c. of the blood, add 15 c.c. of about 20 per cent. solution of K_4FeCy_6 and 2 c.c. of acetic acid, mix and let stand.

(*b*) To 1 c.c. of the blood, add 4 c.c. of water, and 15 c.c. of a 1 per cent. solution of tannin, mix and let stand.

17. **Hematin.** — Hemoglobin, when heated with alkalies or acids, cleaves into a proteid, and a coloring matter called *hematin*. The following experiment illustrates the formation of hematin: To about 10 c.c. of diluted blood (1 : 5) add 1 c.c. of NaOH, and heat until the light red solution turns greenish brown. On spectroscopic examination the whole spectrum is absorbed except the red.

On adding two or three drops of $(NH_4)_2S$, or of Stoke's solution, these absorption bands decrease, and there appear two bands resembling the oxyhemoglobin bands, but a little farther to the right hand, the bands of *reduced hematin* or *hemochromogen*.

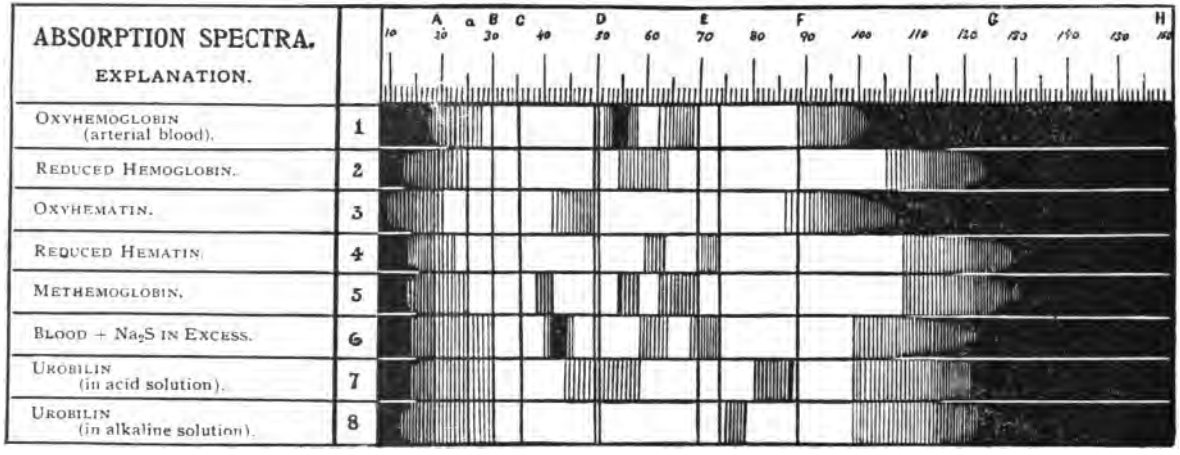
18. **Hematoporphyrin.** — To 10 c.c. of H_2SO_4 in a test-tube add about five drops of blood, agitating after adding each drop. It forms a wine red solution. Dilute with three or four parts of water, and examine with the spectroscope. It shows a narrow band to the left and a broad band to the right of the position occupied by the left-hand band of oxyhemoglobin.

Preparation of Blood Plasma. — This may be prepared in a somewhat diluted form, by drawing the blood into one fourth its volume of a saturated solution of $MgSO_4$ or an equal volume of Na_2SO_4, setting the mixture on ice until the corpuscles settle out and siphoning off the clear plasma.

Potassium oxalate solution (3 grms. dissolved in 50 c.c. of water to 1 liter of blood), may be used to prevent coagulation. The plasma may be separated by the centrifugal machine, if desired. Blood plasma contains three proteids, viz.: serum, albumin, serum globulin (paraglobulin) and fibrinogen.

Blood serum, may be prepared by drawing the blood into a clean tall jar. Place the jar on ice, for 36 to 48 hours, or until the clot has contracted well and expressed a clear yellow serum. This yellow liquid is blood serum, and contains the albumin and globulin but little or no fibrinogen. Ascitic fluid or pleuritic effusion may be employed for the following tests, instead of blood serum.

TABLE OF THE ABSORPTION SPECTRA.



21. Heat a portion of the serum to boiling. It coagulates.

22. Saturate 10 c.c. of the serum with $MgSO_4$ and let set a half hour. The *globulin* precipitates. Filter, and saturate the filtrate with $(NH_4)_2SO_4$ or $ZnSO_4$. The precipitate is *serum albumin*.

23. To a portion of the serum, or transudate, add an equal volume of a saturated solution of $(NH_4)_2SO_4$. Globulin precipitates. Filter off the precipitate, and try its solubility in 1 per cent. NaCl solution. Boil this salt solution to show the presence of globulin in it.

CLINICAL EXAMINATION OF BLOOD.

Hammerschlag's Method of Determining the Specific Gravity.— Prepare a mixture of chloroform and benzene, of about the sp. gr. of 1050, with the aid of an accurate urinometer. Drop one or two drops of the blood under examination into this mixture from a small pipette. If the drops sink to the bottom of the liquid, add gradually some chloroform and mix the solution well with a glass rod until the drops become suspended. If the blood drops float upon the top of the mixture, add benzene, until they remain suspended in the mixture. Now take the sp. gr. of the mixture which is that of the blood. The normal sp. gr. of human blood lies between 1058 and 1062. Pathologically it may vary from 1025 to 1068, varying directly with the per cent. of hemoglobin.

Estimation of the Alkalinity of Blood. — Löwy's Method.— Draw 5 c.c. of blood from one of the superficial veins of the arm into a 50 c.c. graduated flask, containing 45 c.c. of a 0.25 per cent. solution of ammonium oxalate, which prevents coagulation of the blood. Aliquot portions of the mixture are titrated with a $\frac{1}{25}$ normal solution of tartaric acid (3 grms. per liter), removing a drop of the solution, from time to time, on a glass rod, and trying the reaction of this drop with lacmoid papers or sensitive litmus paper previously wetted with saturated NaCl solution. One c.c. of the $\frac{1}{25}$ normal solution of tartaric acid corresponds to 0.0016 grms. of NaOH. Suppose, for example, that 20 c.c. of the mixture, containing 2 c.c. of blood, required 4 c.c. of the acid solution to produce a neutral reaction on the lacmoid paper. The alkalinity of these 2 c.c. of blood corresponds to $4 \times .0016 = .0064$ grms. of NaOH. One hundred c.c. of blood would correspond to $50 \times .0064 = 0.320$ grms. of NaOH.

The alkalinity of the blood of healthy adults corresponds to about .300 to .350 grms. NaOH for 100 c.c. of blood.

Estimation of Hemoglobin.— This is usually done by comparison of the color of the blood, either direct or after dilution, with a standard

color previously prepared. A number of instruments have been invented for the purpose, the chief ones being those of Gower, Malassez, Hayem, Fleischl, Dare and Tallquist. The three last mentioned are those most used at the present time, and of these Dare's instrument is the easiest to use and is less liable to error than Fleischl's because the blood is examined directly, or without dilution. The method of Tallquist has not been in use long enough to have established its claims to accuracy, although it is inexpensive, requires little skill and is said to be reasonably accurate.

Fleischl's Method. —The **hemometer** of Fleischl consists essentially of a round cell, divided by a partition into two compartments, having a glass bottom, and a movable glass cover. This cell is supported on a stand, similar to the base and stage of a microscope, provided with a white opaque plate, in the position of the mirror of the microscope. The cell, above mentioned, is so placed upon the stand that one compartment is illuminated with white light from the white plate, and the other with red light, received through a glass wedge, colored with a red pigment, and made to move along under the cell by a mill-head. The depth of color varies with the thickness of the glass brought under the cell, and a scale indicates the per cent. of the normal amount of hemoglobin in the blood under examination, taking normal blood as 100.

FIG. 1.

To use the instrument, fill both compartments of the cell, nearly full of water. Prick the lobe of the ear of the patient, and place one end of the capillary pipette to the drop and let it fill by capillary attraction, clean off any blood on the outside of the pipette and at once wash it out in the white compartment, taking care that the blood is all washed out of the pipette. This part of the process requires great care, and must be done quickly to prevent coagulation of the blood in the pipette. The pipette should be cleaned with water, then alcohol and finally with ether. Both compartments are then filled with water, taking care not to allow any of the contents of the compartment containing the blood to run over into the other compartment. The cell is now covered by the circular glass cover, so as to

exclude air bubbles, and the color of the two compartments is compared. If not alike, the wedge is moved until the two are the same, and the percentage of normal hemoglobin read off. The real percentage of hemoglobin in normal blood is about 13.7. Therefore, to find the per cent. of hemoglobin in the blood under examination take 13.7 per cent. of the readings of the hemoglobinometer.

The Method of Dare. — The principle of this instrument is similar to that of Fleischl, except that the blood is drawn by capillary attraction into a space between two pieces of glass, M, and examined at once. The comparison color is a colored circular glass wedge which can be rotated so as to imitate the color of the blood. The blood and the glass are viewed through a telescope tube, and thus all reflected or other light is cut off. The results seem uniform and accurate, it requires much less skill and is less liable to errors than Fleischl's instrument.

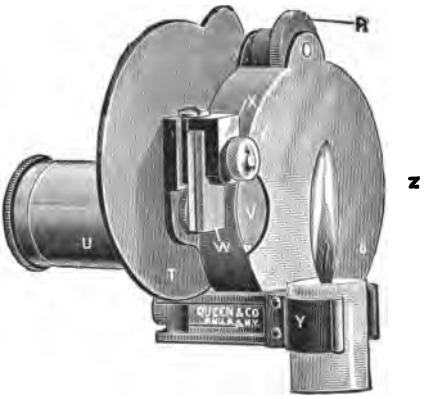


FIG. 2.

Tallquist's paper color scale is intended to be used without any instrument, and may be used by any one at the bedside.

A drop of undiluted blood is soaked into a bit of filter paper of standard quality and compared (by ordinary reflected daylight) with a paper-color scale of ten tints, ranging from ten per cent. to one hundred per cent. The lithographed scale bound up with fifty sheets of the standard filter paper makes a small book which can be easily slipped into the pocket and carried to the bedside.

In making the comparison the blood stain is put against a background of white filter paper beside the color scale, and moved along until the same tint is found. The comparison should be made as soon as the stain has lost its humid gloss, and before it is thoroughly dry. Artificial light cannot be used.

The results are said to be correct to within ten per cent. of the readings on the more accurate instruments.

The estimation of iron in the blood, as a means of estimating the hemoglobin, has not led to results of much value, for the reason that the iron and the hemoglobin do not always run parallel.

MICROSCOPIC EXAMINATION OF BLOOD.

The microscopic examination of blood has become of great practical assistance in the diagnosis of disease. The subject is too extensive to be fully considered here. Special works upon the clinical examination of blood are not wanting, and the student must consult one of these for details of the microscopic examination. We can only consider here the number of corpuscles, without mention of the great variety of corpuscles now recognized in the pathology of the blood.

The number of red corpuscles in a cubic millimeter of the blood of healthy individuals is fairly constant at about 5,000,000 to 5,500,000 in adult males, and 4,500,000 in adult females. The number of white corpuscles, or leucocytes, on the other hand, is subject to rather wide variations even in good health, influenced by age, sex, pregnancy, the process of digestion and the part of the body from which the blood is taken. The number of leucocytes normally found in blood taken from the finger or ear varies between 5,000 and 7,000 in a cubic millimeter. The ratio of the white to the red corpuscles is therefore 1 to 714, to 1 : 1000. Certain exceptional individuals are found whose blood may contain as few as 3,000 or as many as 10,000. Such cases, however, are rarely met with in a state of good health.

An increase in the number of leucocytes is called *leucocytosis*, or *hyper-leucocytosis* and a diminished number is called *hypo-leucocytosis*. Hyperleucocytosis may be physiological or it may be pathological.

Enumeration of the Red Cells.—The determination of the number of red cells is usually made with the Thoma-Zeiss apparatus. This consists of a heavy glass microscopic slide having in its center a cell exactly 0.1 mm. deep between its floor and the cover-glass. The floor of the cell is graduated by cross rulings dividing a portion of it into squares exactly $\frac{1}{400}$ mm. in diameter, and each square therefore underlies a space of $\frac{1}{4000}$ mm.^{3} With the instrument there are two capillary pipettes having a bulb in their upper third, containing a small glass bead to assist in mixing their contents. The pipettes are graduated from the point upward, in tenths from 0 to 1, while above the bulb there is a mark 101. To use the instrument the tip of the finger or the lobe of the ear is thoroughly cleansed, first with soap and water, then with alcohol and finally with ether. The skin is punctured with a needle or scalpel and the exuded blood drawn into the pipette to the mark 0.5 or 1, according to the degree of dilution desired. Care must be taken that the tip of the pipette comes in contact with the blood only and not with the skin. The point of the pipette is now quickly wiped and the blood is diluted with a 3 per cent. solution of NaCl, which is drawn into the pipette to the mark 101.

The contents of the pipette are now thoroughly mixed by shaking. The glass bead assists in the mixing. The contents of the capillary portion of the pipette below the bulb are now cautiously blown out, as this contains only the diluting fluid, and a drop of the mixture from the bulb blown out into the counting chamber, and the cover glass carefully placed upon it so as to exclude all air bubbles. After 3 to 5 minutes the corpuscles will have settled down on the ruled floor of the cell and the counting may begin.

Toison's fluid is often very convenient to use as the diluting fluid instead of the 3 per cent. salt solution, especially when it is desired to count the leucocytes, as these cells are stained by the methyl violet which it contains.

This solution is made as follows:

Methyl Violet	0.025	grm.
Sodium Chloride	1	"
Sodium Sulphate	8	grms.
Glycerin	30	"
Distilled Water	160	"

It is not necessary to work with a very high power objective. A Reichert No. 5 objective answers the purpose well. At least one whole field of 200 squares, and where great accuracy is desired, 400 small squares should be counted. To calculate the number of corpuscles found, divide the whole number of corpuscles counted by the number of small squares containing them. This gives the number of cells in each square. This number multiplied by 4,000 will give the number in 1 mm.^{3} of the diluted blood. This multiplied by the degree of dilution, usually 100 or 200, gives the number in 1 mm.^{3} of the undiluted blood.

Counting the Leucocytes. — To make a reasonably accurate count of the white corpuscles it is best to use the large bore pipette, and a diluting solution of one third per cent. acetic acid, which renders the red cells invisible and leaves the white cells only to be counted.

The drop of blood needed is larger than that used for counting the red cells, and the pipette should be held almost horizontal while filling it, and apply very gentle suction.

Instead of counting separately the field of thirty-six squares, we count the corpuscles in the whole ruled space, and repeat the process with a second drop.

In most cases, and after a little experience, it is possible to count both the red and white cells with the smaller pipette.

In this case it is necessary to count a large number of squares, at least ten times as many as necessary in counting the red cells. To make the process less tedious we may measure the diameter of the field of the lens we are using, and then count a series of these fields outside of the ruled space, moving the stage, after each count of a field, exactly the width of the field. In this way we may count ten or twelve fields on the one slide.

A still better plan is to cut out of a piece of black cardboard a disc just large enough to fit into the tube of the eye piece of the microscope. Then cut a square hole in this of such size that 100 of the ruled squares, or one quarter of a square millimeter, can be seen through it. A number of such fields may then be counted, outside of the ruled space on the slide, taking care in moving the slide so that the cells seen on the extreme margin of one field move to the opposite margin. It is

very convenient to have a mechanical stage for this work, although this is not essential. With skill and experience, the stage can be moved by hand.

The Examination of Dried and Stained Blood. — The study of the structure or variations in the cellular elements of the blood, is generally done with dried and stained cover-glass preparations.

To prepare such specimens for examination, the drop of blood, as it emerges, is touched with the flat surface of a carefully cleansed glass slide, near one end. This slide is pressed upon a second slide, at an angle of about 45 degrees to it, and, drawn from end to end, so as to form a thin, uniform smear. It is best to prepare two or three such smears, to provide for a possible accident to one of them. They are then allowed to dry, either at the ordinary temperature, or hastened by the aid of a gentle heat. The next step is to fix the albumin by heating the preparation on a brass plate, or in a sterilizer oven at a temperature of 110° C. to 115° C. A strip of copper or brass plate about two inches wide and 10 or 12 inches long, supported on a tripod, is heated at one end by a Bunsen burner or alcohol lamp. Determine the point on the heated plate where water boils, by drops of water. Drop the dried cover-glass preparations, face down, on this part of the plate, and allow them to remain about fifteen minutes. After allowing the specimens to cool they are ready to stain.

Another method of fixing the specimen is to place the slide containing the film of blood for a half hour in a mixture of equal volumes of absolute alcohol and ether, then remove them, wash with water and dry.

The most generally useful stains are Ehrlich's triacid stain, Wright's stain, and Goldhorn's polychrome methylene blue, which can be obtained from dealers ready made.

The method of staining is simple. The stain is dropped on the specimen, spread over every portion of the surface with a glass rod, allowed to remain from one to five minutes and then washed off with clean water, dried between filter paper and mounted in Canada balsam.

The examination must be made with the one-twelfth oil immersion lens, with Abbe condenser and wide open diaphragm.

The examination of such specimens will show the different varieties of red and white cells, the malaria plasmodia, etc.

For a description of these varieties of cells and their significance the student is referred to the special works on the subject.

QUANTITATIVE ANALYSIS.

Quantitative analysis, or the accurate determination of the amount of the constituents of a mixture, or the *ions* of a compound, may be carried out **gravimetrically,** or by weight, or **volumetrically,** or by measure.

We can thus easily divide the subject of quantitative analysis into: Gravimetric analysis and volumetric analysis.

In gravimetric estimations the ion, or substance to be estimated, is converted into an insoluble form, which is then separated from other substances present by filtration, washed free of impurities, dried and weighed. In some cases, as in most inorganic ions, the dried

precipitate is ignited in a crucible, the paper burned off and it is then weighed, deducting the weight of the ash of the paper.

In the case of organic or volatile substances, they are collected upon a previously dried and weighed filter paper, and the weight of the paper is then deducted from the final weight of substance and paper. When filtering off a precipitate, in quantitative analysis, the filtrate must come through perfectly clear and transparent. If it does not do so, it must be passed through the paper again, or until it does come through clear, otherwise there will be a loss of the precipitate and the final result will be too low.

In quantitative analysis *the most scrupulous attention to cleanliness and all the details of the process must be adhered to.* Very slight variation from the prescribed details of a process will often vitiate the whole operation and lead to erroneous results. Extreme accuracy in all weighings and measurings are essential to success.

The Balance.—Gravimetric analysis requires a sensitive balance, or one that will weigh to a tenth of a milligramme, and a set of metric weights ranging from 50 grms. to one milligramme. Such a balance is usually called a chemical balance and is inclosed in a glass case for protection. It consists essentially of a beam, moving on knife edges of agate or steel, bearing on plane surfaces of agate or steel and carrying at each end a pan.

When not in use, the knife edges are raised from the planes by a mechanical support worked by a mill-head. *In weighing with such a balance,* the substance is placed upon the left-hand pan and balanced by placing the weights upon the other pan, always handling the weights with the forceps furnished for the purpose. After placing on the pan as much weight as thought to be sufficient to balance the substance to be weighed, the support is slowly lowered and the beam is allowed to swing. Weights are now added or removed until equilibrium is established and the pointer swings equally on both sides of the center.

The beam is then supported, before removing the weights, and the weights counted while removing them to their places in the box and the weight at once recorded. The weighing of most substances should be done as quickly as possible, because of changes in weight by absorption of water from the air. Liquids should be weighed in a bottle or other closed vessel to prevent evaporation during the weighing.

Drying Precipitates.—Every substance to be weighed must be dried to free it from water. This requires great care and considerable consumption of time, especially in dealing with organic substances, or those to be weighed with the filter paper.

The drying of precipitates is usually done in an *air oven* or a *water oven*, or sometimes it is begun in the former of these and completed in the latter.

While cooling, the substance is protected from moisture by placing it in a desiccator or vessel containing some H_2SO_4 or dry $CaCl_2$ to keep the air dry. This precaution must always be observed.

Few gravimetric processes are used in clinical work, owing to the time consumed. For this reason we shall not give a detailed description of these processes.

VOLUMETRIC ANALYSIS.

Because of the rapidity and ease with which quantitative analyses can be made by means of volumetric solutions, this method is almost universally adopted in clinical work. As most of the quantitative processes described in the following pages of this book depend upon the use of volumetric solutions, it is necessary for the student to understand their preparation, the principles of their use, and the apparatus employed.

By **volumetric analysis** is meant the quantitative estimation of a substance, by adding to it a measured volume of liquid containing a known amount of the reagent, and depending upon an indicator to show when the precipitation or reaction is completed. This process is called **titration.**

Volumetric analysis requires:

1. A graduated vessel, from which accurately measured portions of the reagent liquid may be delivered, called a **burette.**

2. A solution of the reagent, of known chemical power, and called a **standard** or **volumetric solution.**

3. The decomposition produced by the reagent in the standard solution, with the substance to be estimated, must be either such that by itself or by the aid of an indicator *its completion is unmistakably evident to the eye*, and thus the quantity of the substance with which it has reacted may be calculated.

Measuring Instruments. — The apparatus needed for the volumetric methods are usually few and inexpensive. An expensive balance is not essential for the methods to be mentioned below, as the standard solutions are to be had of wholesale druggists or chemical dealers, or can be prepared by any competent pharmacist. A **burette** is a graduated tube holding from 25 c.c. to 100 c.c., and provided with a stop-cock at one end and terminating in a tube of small caliber. The most convenient burette is one of 50 c.c. capacity, and graduated to 0.1 c.c. (See Fig. 3.)

Pipettes (Fig. 5) are graduated tubes, drawn down at one end to a small opening, and intended to be filled by suction at the upper end, after which the *dry* finger is pressed upon the upper end to control the flow. A *graduated flask* is a glass flask having a narrow neck, upon which is a mark denoting its capacity when filled to this mark. The most convenient sizes for general purposes are 50 c.c., 100 c.c., 250 c.c., and 1000 c.c. It is convenient to have a 10 c.c. and a 25 c.c. graduated cylinder or hydrometer jar.

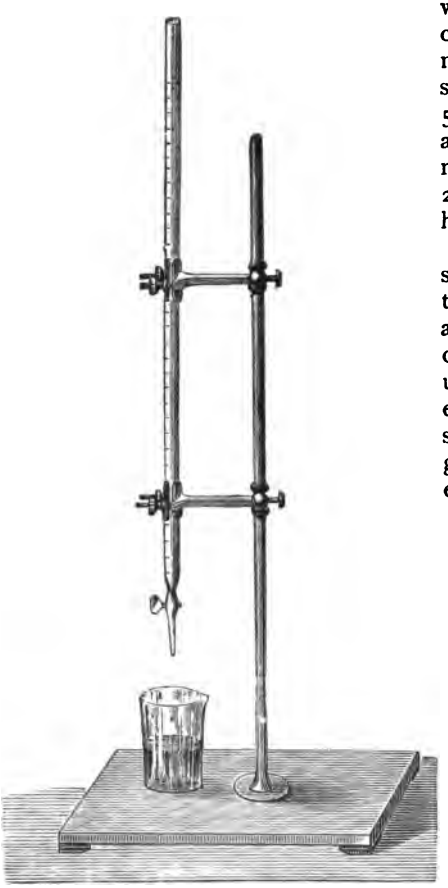


FIG. 3.—GRADUATED BURETTE.

Standard Solutions. — A standard solution is one containing a known amount of an active chemical substance or reagent in a measured volume of liquid. It is usual to express the strength of such solutions in the number of grams of the active ingredient to the liter, or 1000c.c.

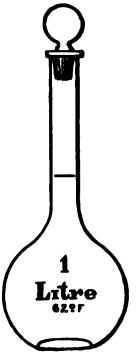


FIG. 4.

FIG. 5.

of the solution. *Titer* is a term used to indicate the strength of one solution as compared with that of another, or the ratio of the former to the latter. Standard solutions may be *empirical* or *normal*.

A **normal solution** is one containing the chemical equivalent of one atom of hydrogen, or any other monad ion, expressed in grams, dissolved in one liter. From this it follows that a liter of a normal acid will contain 1 grm. of basic hydrogen ions, and of a normal alkali will contain the chemical equivalent of 1 grm. of these H ions.

Thus: one molecule of HCl is the chemical equivalent of the ions Na, Ag, Br, I, or H.

$$HCl + AgNO_3 = AgCl + HNO_3.$$
$$HCl + NaOH = NaCl + H_2O.$$
$$HCl + NaI = NaCl + HI.$$

The molecular weight of HCl is $35.5 + 1 = 36.5$. The normal solution of HCl will then contain 36.5 grm. by weight (the gram molecule) of HCl to the liter of which 1 grm. is H ions and 35.5 grms. are Cl ions. The chemical equivalent of H_2SO_4 in Na or H is twice that of HCl, or one molecule of H_2SO_4 will neutralize Na_2. The molecule of H_2SO_4 is represented by $2 + 32 + 64 = 98$. Of 98 grms. of the acid 2 grms. are H ions. The normal solution should contain but 1 grm. of H ions. Hence the normal solution will contain one half its molecule, expressed in grams, dissolved in one liter, or, $98 \div 2 = 49$ grm. A normal solution of oxalic acid, $H_2C_2O_4.2H_2O$, will also contain one half its molecular weight, dissolved in a liter. A normal solution of sodium hydroxide will contain the molecular weight expressed in grams, because NaOH is the chemical equivalent of H, Cl, Br, I, or any other monad ion.

A decinormal solution is one containing one tenth the active ingredient of the normal solution, or one tenth the chemical equivalent of hydrogen, expressed in grams, dissolved in a liter. It is often made from the normal solution by diluting 100 c.c. of the latter to a liter with distilled water. A $\frac{1}{50}$ normal and a $\frac{1}{100}$ normal solution or a centinormal solution are sometimes used. A normal solution is frequently expressed thus: $\frac{N}{1}$; a decinormal, $\frac{N}{10}$, or $\frac{1}{10}N$; and a centinormal, $\frac{1}{100}N$, or $\frac{N}{100}$.

An *empirical standard solution* is one of undetermined strength, which is balanced with another known solution by comparative titrations. Its strength or *titer* is compared to another solution by trial.

Preparation of Normal or Standard Solutions. — The greatest possible care and accuracy must be used in the preparation of these solutions. A slight error in preparing them will vitiate all analyses in which they are used.

When the substance to be employed in making up a solution is a solid, the calculated quantity of the purest obtainable substance is weighed out on an accurate sensitive balance, and dissolved in an accurately measured quantity of distilled water. The purity of the substance is usually determined by appropriate tests or by recrystallization.

The solution when completed is then "*standardized*" by titrating it against some solution of known composition. Thus a solution of $AgNO_3$ is usually "checked" or "standardized" by carefully weighing out a portion of fused sodium chloride and titrating this with the silver solution. By a simple calculation the "titer" of the silver solution is determined.

From a normal solution we may make a decinormal solution by measuring out 100 c.c., putting this into a liter flask and filling to the mark on the neck with distilled water.

Factors.—In the use of a standard or volumetric solution, it is necessary to know or be able to calculate the ratio of the solution to the substance under estimation. This ratio is generally spoken of as the *factor*. The factor for normal solutions is easily found, if it be remembered that one c.c. of any normal acid solution will exactly neutralize 1 c.c. of a normal alkali. To calculate the factor for any solution, write the equation, representing the reaction, and then make the proportion: *The formula of the substance known* (the reagent) *is to the formula of the substance to be estimated, as the weight of the reagent in 1 c.c. of the solution to X, the weight of the substance to be estimated, corresponding to 1 c.c. of the standard solution.*

For example, suppose it is desired to estimate NaCl, with a normal solution of $AgNO_3$. The reaction is represented as follows:

$$AgNO_3 + NaCl = AgCl + NaNO_3$$

The molecular weight of $AgNO_3 = 170$, *i. e.*, one liter of the normal solution contains 170 grms. and 1 c.c. contains .170 grms. or 170 mgrms. $NaCl = 58.5$. Substituting these numbers in the above equation we have:

$$170 : 58.5 :: 0.170 : x = 0.0585 \text{ grms.}$$

Therefore 1 c.c. of a normal solution of $AgNO_3$ will precipitate 58.5 mgrms. of NaCl. One c.c. of a decinormal solution will precipitate 5.85 mgrms. of NaCl.

Example: What is the factor in NaH_2PO_4 of 1 c.c. of decinormal NaOH? One c.c. of $\frac{N}{10}NaOH$ contains 0.004 grms. NaOH.

Equation:

$$NaOH + NaH_2PO_4 = Na_2HPO_4 + H_2O$$

$$NaOH = 40. \quad NaH_2PO_4 = 120.$$

$$40 : 120 :: 0.004 \text{ grms.} : x = 0.012 \text{ grms.}$$

For some purposes it is more convenient to have a standard solution of such strength, that 1 c.c. of it will correspond to a known and convenient amount of the substance to be estimated. Such a solution is sometimes called an **empirical-standard solution.** Thus the alkaline-copper solution, known as Fehling's solution, is prepared of such strength that 1 c.c. will be reduced by .005 grms. of dextrose. In some cases, where the reaction is complicated the factor is determined by experiment.

Let it be desired to prepare a solution of NaOH of such strength that 1 c.c. of it shall neutralize 10 mgrms. of HCl. Equation

$$HCl + NaOH = NaCl + H_2O$$

$$36.5 : 40 : : .010 : x = .0109 \text{ gms.}$$

That is, each c.c. of the NaOH solution, must contain .0109 grms. of NaOH.

Example. — A decinormal solution of NaCl is prepared by dissolving exactly 5 85 grms. of fused pure NaCl, in water sufficient to make a liter at the temperature at which the flask is graduated. One c.c. of this solution contains .00585 grms. of NaCl.

Measure out exactly 10 c.c. of this solution into a small beaker, add 40 c.c. of distilled water, three drops of a 10 per cent. solution of K_2CrO_4 for the indicator and run in the $AgNO_3$ solution until the yellow color of the solution changes to orange, showing that the chlorine is all precipitated and the silver is beginning to form silver chromate. The first change of tint is to be carefully noted and the reading taken. If exactly 10 c.c. of $N/10$ solution of $AgNO_3$ are required to produce the change of tint of the solution, the solution is correct.

But suppose 9.5 c.c. of the $AgNO_3$ solution produces the change, showing that the solution is too strong. We can calculate the factor of the $AgNO_3$ solution in NaCl by the following proportion :

$$9.5 : 10 : : .0585 : x = .00615.$$

That is, 1 c.c. of the $AgNO_3$ solution will precipitate .00615 grms. of NaCl. If we desire to make this solution decinormal we may do so by dilution, by the proportion :

$$9.5 : 10 : : 100 \text{ c.c.} : x = 105.2 \text{ c.c.}$$

That is, we must add 5.2 c.c. of distilled water to every 100 c.c. of the solution.

Preparation of Normal Hydrochloric Acid. — This solution is prepared from the commercial concentrated pure acid, sp. gr. 1.20, or the U. S. P. acid, sp. gr. 1.16. The first contains about 40.8 per cent. of HCl and the latter 31.9 per cent.

As the normal solution contains 36.5 grms. of HCl per liter, or 3.65 per cent., we can approximate the proper strength by diluting 114 c.c. of the U. S. P. acid with sufficient water to make one liter.

32 per cent. : 3.65 per cent. : : 1000 c.c. : $x = 114$ c.c. It is not safe to depend upon the strength of the solution made in this way.

We, therefore, make the solution a little stronger than that desired, and then check it or standardize it with some base of known weight and composition.

Method of Procedure.—About 125 c.c. of the commercial strong acid is measured into a liter flask, water added to the mark on the neck and the contents of the flask are well mixed. A second approximate solution is made by dissolving 50 grms. of the purest commercial NaOH in water enough to make one liter. The exact relative strength of these two solutions is determined with the greatest possible care by a series of titrations. This is done by measuring out 10 c.c. of the acid into a small beaker, adding about 40 to 50 c.c. of water, three drops of phenol-phthaleïn solution, and then run in the NaOH solution from a burette, stopping when the faintest permanent pink color is produced. This process is repeated a number of times until the true reading is determined.

Weigh out into a beaker a convenient amount of pulverized crystalline Iceland spar ($CaCO_3$), previously well dried in a water oven, say 0.5 grm. Add a small quantity of water and then run in an excess of the acid, say 15 c.c., and warm the solution on a sand-bath to expel the CO_2. The reaction may be represented by the following equation:

$$2HCl + CaCO_3 = CaCl_2 + H_2O + CO_2.$$

The excess of HCl present is now determined by "back titration" with the NaOH solution, using phenol-phthaleïn or methyl-orange as the indicator. Suppose, for example, that the relative titrations of the two above solutions have shown that 10 c.c. of the acid required 11.0 c.c. of the NaOH solution, and therefore 1 c.c. of the latter solution is the equivalent of .909 c.c. of the acid. Suppose, also, that the back titration of the excess of acid left after dissolving the 0.5 grm. of $CaCO_3$ in 15 c.c. of the acid, required 6.5 c.c. of the NaOH solution. Then, $.909 \times 6.5 = 5.9$ c.c., which represents the number of cubic centimeters of acid in excess. The difference between this and the acid added ($15 - 5.9 = 9.1$) gives the number of c.c. required to combine with 0.5 gms. of $CaCO_3$. The amount of HCl in the 9.1 c.c. of acid is calculated by the following proportion:

$$(CaCO_3 = 100.08) : (2HCl = 72.92) : : 0.5 : x = .3643 \text{ grms.}$$

$.3643 \div 9.1 = .040$ grms. of HCl in 1 c.c. of the solution, or 40 grms. per liter.

To make this solution of normal strength it is necessary to dilute it with water until it contains 36.43 grms. per liter. The volume to which each 100 c.c. of this acid is to be made up may be found by the following proportion:

$$36.43 : 40 : : 100 : x = 109.8.$$

To each liter of the solution 9.8 c.c. of water are to be added. It is sometimes more convenient to use pure sodium carbonate, instead of Iceland spar, as a means of standardizing the acid.

Pure sodium carbonate may be prepared from the commercial bicarbonate, after washing it with cold distilled water until the wash-water is free from chlorides or sulphates, and drying and igniting in a crucible at a dull red heat for fifteen minutes. A convenient amount of this freshly ignited $NaCO_3$ is dissolved in water, an excess of acid added and the excess of acid determined exactly as with the Iceland spar.

Preparation of a Normal of Sodium Hydroxide Solution. — Having prepared and standardized a normal acid, the normal alkali solution may easily be standardized with it.

About 50 grms. of the best commercial NaOH is dissolved in a liter of distilled water, its strength determined with the acid, and then diluted as in the case of the acid above described.

Or, we may proceed as follows: The solution is prepared as above and titrated against a normal oxalic acid solution. To prepare this acid solution weigh out exactly 63 grms. of pure crystallized oxalic acid, $H_2C_2O_4.2H_2O$, taking care that it is free from any evidence of extra moisture or efflorescence of the crystals, and dissolve this in one liter of distilled water. Weigh out about 50 grms. of best commercial NaOH, and dissolve this in water, cool, pour into the 1-liter flask and make up to one liter.

To "*standardize*" this solution proceed as follows: Measure out into a beaker 10 c.c. of the solution, add three drops of a 1 per cent. alcoholic solution of phenolphthaleïn, and run in the oxalic acid from a burette until the pink color is just discharged. Suppose 12 c.c. of the acid solution be required to do this. Then 10 c.c. of the NaOH solution contain the amount of NaOH that should be contained in 12 c.c.; 100 c.c. will contain the amount that 120 c.c. should contain. To make a normal solution of this solution, 20 c.c. of water must be added to every 100 c.c. We may calculate the amount of water to be added to any number of cubic centimeters by the following proportion:

$$10 : 12 : : \text{any measured volume} : x.$$

Or we may calculate the number of cubic centimeters to be used to make 1000 c.c. by the proportion:

$$10 : 12 : : x : 1000.$$

$$10 \times 1000 = 10{,}000. \quad 10{,}000 \div 12 = 833.3 \text{ c.c.}$$

A decinormal solution is prepared from the normal solution by dilution with water.

Indicators. — In volumetric analysis some coloring matter is usually employed to indicate when the reaction is complete either by a change of color or by a precipitate. An indicator usually shows when sufficient of the reagent has been added by a change of color. The indicators most frequently used are litmus, cochineal, turmeric, hematoxylin, and Brazil wood from the vegetable kingdom, and a variety of artificial colors obtained from coal tar. Of these, some are of general utility, while others are suitable for special titrations only. The following are those most often used in general practice, with their indications, with acids and alkalies:

	With Acids.	With Bases.
Litmus (aqueous solution),	Red.	Blue.
Lacmoid (0.2 grms. in 100 c.c. alcohol),	Red.	Blue.
Cochineal (3 grms. in 250 c.c. 25 per cent. alcohol),	Yellowish-red.	Violet.
Hematoxylin (0.5 grm. in 100 c.c. alcohol),	Red.	Blue.
Phenol-phthaleïn (1 grm. in 100 c.c. alcohol),	Colorless.	Red.
Rosolic acid (1 grm. in 100 c.c. 60 per cent. alcohol),	Yellow.	Rose-red.
Alizarin (0.5 grm. in 100 c.c. water),	Yellow.	Violet-red.

Dimethyl amido-azobenzene (0.5 grm. in 100 c.c. alcohol),	Red.	Yellow.
Tropeolin 00 (0.5 grm. in 100 c.c. 50 per cent. alcohol),	Orange.	Yellow.
Congo Red (0.5 grm. in 100 c.c. 10 per cent. alcohol),	Blue.	Red.
Methyl orange (0.05 grm. in 100 c.c. distilled water),	Yellow.	Red.
Brazil wood (watery solution).		

Of these colors, lacmoid, cochineal, congo red and tropeolin 00 are more sensitive towards bases than toward acids. The remainder are more sensitive towards acids.

The following are sensitive to CO_2 in solution: Cochineal, Congo red, lacmoid, methyl orange.

Dimethyl amido-azobenzene is sensitive to mineral acids, but is not very sensitive to organic acids. It is used to distinguish these two classes of acids.

Phenol-phthaleïn is not sensitive to ammonia.

The *quantity of the indicator* to be used is just enough to give the solution a decided tint. An excess of color destroys its sensitiveness. From 3 to 5 drops is the quantity of the above solutions usually employed.

Volume of the Solution. — The relative volume of the water used in volumetric operations exerts a material influence upon the results, especially in the presence of very sensitive indicators.

The solution should not be very concentrated, nor too dilute. The proper volume of a solution for titration is between 50 and 100 c.c.

Test papers are prepared as follows:

The best Swedish or Schleicher and Schüll quantitative filter paper is immersed in a fairly strong solution of the indicator until thoroughly saturated. The paper is then removed from the solution, drained for a few minutes and dried either upon a glass plate or by suspending from a stretched cord, reversing the position occasionally to distribute the color evenly over the surface. If the above filter paper is not at hand any good white filter paper, free from starch, may be prepared by immersing it in pure HCl for several hours, washing in warm water, then with dilute ammonium hydroxide solution and finally washing thoroughly in distilled water until the washings do not react alkaline with litmus paper.

The test papers most used are litmus. The litmus is rubbed up in a mortar with alcohol as long as it extracts any red color. It is then rubbed up with distilled water and the blue solution filtered.

A portion of this solution is made feebly acid and red papers are prepared from this, while blue papers are prepared from the blue solution.

The other test papers often used are turmeric, methyl orange, Congo

red, phenol-phthaleïn, hematoxylin, Brazilian cochineal, starch, starch and potassium iodide and lead acetate papers.

Test papers are useful in titrations of colored solutions, or of solutions containing precipitates which would obscure the color of the indicator, or in liquids which destroy the colors by oxidation or reduction.

They are used by the removal of a drop of the solution on a glass rod and moistening the paper with it.

THE URINE.

The urine is an excretory fluid thrown off by animals. It is partially filtered from the blood by the kidneys, and partly elaborated by these organs from waste materials found in the blood. It is composed of a watery solution of certain inorganic salts and nitrogenous principles which are of no further use to the body. As will be seen from the table at the end of this chapter, human urine is not a liquid of uniform composition, but subject to very considerable variations. These variations may be physiological, or they may be indicative of diseased conditions, and a knowledge of them is essential to a correct diagnosis of many diseases.

General Physical Properties. — Normal urine, when fresh, is a clear, amber-colored, transparent liquid, having a peculiar, aromatic, characteristic odor, a bitter, saline taste, a distinctly acid reaction, and a specific gravity of from 1018 to 1022. The average specific gravity is generally given as 1018 to 1020. When it is kept in a clean vessel and away from contact with air, it will undergo but slight changes in several days.

Composition.—The urine is chiefly a solution of urea and certain organic and inorganic salts, holding in suspension epithelial cells and mucus. The composition will be found in the table at the end of this chapter, with the chief variations met with in diseased conditions and their significance. The urine, like milk and other animal fluids, is not of constant composition. It is influenced by the amount of water and other fluids taken; by the temperature of the skin; by the emotions; by the blood pressure, local or general; by the amount of work done, the time of day, the age, the sex, the influence of medicine, etc.

Quantity.—The **quantity** of urine passed in twenty-four hours varies considerably. The average daily quantity passed by a healthy adult weighing 150 pounds is from 1,200 to 1,500 c.c., or about 40 to 50 fluidounces. The quantity of total solids contained in this is about 60 grms., or 1,000 grs., and about one half of these solids is composed of urea. Females pass less urine and solids than males.

The variations in the quantity will be found in the table below.

An **increased quantity** of urine is passed after drinking large quantities of fluids — **urina potus**. This is especially noted after drinking water containing no salts, or after drinking beer, wine, coffee, tea, etc., diuretic medicines, such as digitalis, squills, strophanthus, spirits of nitrous ether, juniper, urea, etc.

The quantity of water secreted by the kidneys is largely dependent upon the blood pressure brought to bear upon the glomerular tufts of capillaries. The more active the circulation, the more active the secretion of urine. This is somewhat modified by the presence or absence in the body of certain substances which stimulate the epithelial cells of the tubules, as urea, sugar, salts of alkaline metals, etc.

CLINICAL SIGNIFICANCE OF VARIATIONS IN THE VOLUME OF URINE IN 24 HOURS.

		DISEASES.		REMARKS.
Increased Volume.	Polyuria ($V+$)	Renal Affections.	Interstitial Nephritis.	2 to 4 liters.) (66 to 130 oz. Trace of Albumin.
			Amyloid Degeneration.	2 to 6 liters. Albumin.+
		Nutritive Disorders.	Diabetes Mellitus.	Glucose, Acetone. Diacetic Acid.
			Diabetes Insipidus (Phosphaturia).	Urea+ Uric Acid + Phosph. Acid. +
		Nervous.	Hysteria.	
		Essential.	Epilepsy. Dementia.	Toxicity Lessened.
Decreased Volume.	Oliguria. ($V-$)	In Febrile Diseases.	Dermatitis. Gout. Toxic Conditions.	Acidity increased.
		In Chronic Affections.	Of the heart.	Albuminuria.
			Of respiratory organs. Of the kidneys. Of the liver and uterus.	See microscopic examination.
	Suppression or Anuria.	Calculus or tumor.		Confirm by the quantity of urea. Albuminuria or hematuria.
		Certain forms of nephritis.		
		Nervous anuria.		
		Accidental anuria.		After severe burns, or after anesthetics.

The Color and Transparency. — In health, the color is usually a light amber. In general, the greater the quantity, the lighter the color; and the smaller the quantity, the darker the color. As the color deepens by concentration, it becomes more reddish. The color, as well as the quantity, is subject to great variations, even in health. It may vary from almost as clear as water to a dark yellowish-red, according to the degree of concentration. After drinking large quantities of fluids, the quantity is very much increased and the color is light. After severe sweating, or in abstinence from drinking, it becomes concentrated and darker in color. The normal color of the urine is due to several more or less closely allied pigments, the chief of which are **urobilin** and **uroxanthin.** These coloring matters are probably derived from the biliary coloring matters. The abnormal coloring matters are chiefly those of the blood or bile, melanin, hemoglobin, and coloring matters due to medicinal substances and certain vegetables. An excess of the normal pigments of the urine may be expected in febrile conditions, and in diseases in which the blood-cells are undergoing rapid destruction. Urobolin, when it exists in excessive quantities, colors the urine a dark brownish-red, even without concentration, and the foam of such a urine is of yellow or yellowish-brown color. Febrile urine is reddish-brown. Bloody urine is red. The color is smoky in acute nephritis, after $KClO_3$, C_6H_5OH, creosote, AsH_3, etc. Yellow or green indicates bile (icterus). A dirty blue color is sometimes seen in cholera, typhus, and from indigo. A white color may be due to chyle, pus or phosphates.

Effects of Drugs, etc. — Dark: C_6H_5OH, creosote, $KClO_3$, tar, turpentine, terpin hydrate, salol, quinine (sometimes), phenocoll, naphthalene, antipyrin. Red: Analgen, cascara, sulphonal (sometimes violet-red), madder, aloes, and magenta. Yellow: Thalline, santonin, cascara, rhubarb, senna, chrysophanic acid, and gamboge. Blue: Indigo, methylene blue, and pyoctannin. Brown or brownish-black urine is observed in patients with melanotic tumors. The coloring matter in this case is melanin.

Transparency. — Normal urine is transparent, containing only a slight flocculent cloud of mucus, visible after standing a few minutes. Turbid urine is pathological. It is usually turbid in all diseases of the urinary bladder, from the excessive amount of mucous and epithelial elements, and because the urine in this condition readily undergoes alkaline fermentation in the bladder, when the *earthy phosphates* are precipitated as a white sediment. In fevers, the quantity of urine is occasionally so small that the urates separate even in the bladder, and especially is this the case in certain diseases of children, where

oxidation is deficient, as in capillary bronchitis and pneumonia. Admixtures of *blood*, *pus*, and *chyle* make the urine turbid. The most striking turbidity is produced by the admixture of *chyle*, which gives it a milky-white appearance. Here the milky appearance is due to an admixture with the urine of emulsified fat and imperfectly dissolved proteids, usually globulin. Many times which are clear when passed become turbid on standing, from the separation of the acid urate of sodium or ammonium. The turbidity of alkaline urine has already been mentioned. All urines become turbid on standing for a few days, from the appearance of swarms of *bacteria* in the solution. Such turbidity cannot be separated by filtration through paper. They can be removed by shaking the urine with some insoluble powder and repeated filtration. Powdered glass, silica, or talcum answers the purpose. Another method is to add a few drops of Na_2HPO_4 and then a slight excess of NH_4OH to precipitate the earthy phosphates, and then filter.

Specific Gravity. — This varies from 1015 to 1028, according to the degree of dilution or concentration. Pathological urines may vary from almost that of water to 1050. As a rule, the urine of Bright's disease is of low specific gravity, while in diabetes mellitus, and in all acute fevers, it is of high specific gravity. The specific gravity of urine is generally determined by the *urinometer*, which is a small hydrometer graduated to include the variations in specific gravity usually found in urine. It is usually graduated so that only the last two figures of the specific gravity appear upon the stem, and to read correctly at 60° F. If the temperature is above 60° F., it will be sufficiently accurate for clinical purposes to add one degree in specific gravity for every 3° C. (5.4° F.) in temperature — *i. e.*, if it read 1018 at 80° F., it would read 1022 at 60° F. The ordinary urinometers of the market are apt to be unreliable. It is best, therefore, to test the instrument by careful determinations of the specific gravity of solutions of common salt, with the specific gravity flask, and compare the readings of the urinometer with these determinations.

The urinometer is used as follows: The urine is placed in the upright jar, or cylinder, wide enough and deep enough to allow the instrument to float freely. When it has come to rest, the surface of the fluid in the jar is brought to the level of the eye, and the reading taken at the lower edge of the meniscus formed by the upper surface of the urine. The mark on the instrument which is cut by this line, and which can be distinctly seen in clear urine, is taken as the correct reading. If the urine be turbid, this method can not be employed, as the reading will be more or less uncertain. Should the quantity of urine at hand be not enough to float the urinometer, it may be diluted

with an equal volume of water, the specific gravity taken, and the last two figures multiplied by two, to get the true specific gravity.

Clinical Significance of Specific Gravity, or Density. — The specific gravity is, in general, a guide to the secreting power or efficiency of the kidneys, the activity of the circulation and the activity or condition of bodily metabolism. When the kidneys are diseased the urine is apt to be of low specific gravity though not necessarily so, as other conditions as fever, cardiac disease, etc., modify it.

Specific gravity, when taken with the quantity passed, is of use in calculating total solid matter passed. The following table gives the number of grains of solid matter in a fluidounce of urine of the given specific gravity:

Specific gravity of	1005	gives	5.10	grains of solids in	1 oz.
"	"	1010	"	10.20	"	"	1 oz.
"	"	1015	"	15.30	"	"	1 oz.
"	"	1020	"	20.40	"	"	1 oz.
"	"	1025	"	25.50	"	"	1 oz.
"	"	1050	"	51.00	"	"	1 oz.

The amount of solid matter excreted in twenty-four hours by an average adult man is about 60 to 65 gms., or 1000 grs. From the above table it is seen that the last two figures of the specific gravity very nearly express the number of grains in each fluidounce of normal urine. By multiplying these two figures by the number of ounces passed in twenty-four hours, we obtain approximately the number of grains of solid matter passed in twenty-four hours. Various experimenters have sought a *coefficient* or formula by which to calculate the result in grams per liter. Trapp obtained the number 2; Loebisch, 2.2; Christison and Haeser, 2.33. Thus, if a given urine has a sp. gr. of 1020, it will contain, approximately, $20 \times 2.2 = 44$ gm. per liter. If the quantity of this same urine be 1200 c.c. per twenty-four hours, then the total solids will be found by multiplying the above result by the number of liters — $44 \times 1.2 = 52.8$. The most striking variations from the normal solids will be found in diabetes mellitus and in the various forms of nephritis.

Urine is pathological if we observe:

1.	Specific gravity	high,	color	high,	quantity	small.
2.	"	"	"	pale,	"	large.
3.	"	"	low,	"	high,	"	large.
4.	"	"	"	"	very pale,	"	small.

If urine is persistently below 1015, suspect albuminuria and nephritis; if below 1008, suspect albuminuria or hydruria. If persistently

above 1025, color pale, quantity increased, it indicates diabetes. If persistently above 1025, color high, quantity diminished, suspect fevers, some cardiac weakness, or excess of uric acid.

Examples.

CASE 1. — Quantity in twenty-four hours, 900 c.c. ($\frac{9}{10}$ of a quart); specific gravity, 1030 to 1035; color high: Severe fever.

CASE 2. — Quantity, 4800 c.c. (5 quarts); specific gravity, 1025 to 1035: Diabetes mellitus.

CASE 3. — Quantity 3500 c.c. (3½ quarts); specific gravity, 1015: Diabetes insipidus or interstitial nephritis.

CASE 4. — Quantity, 600 c.c.; specific gravity, 1010: Chronic nephritis.

A more exact method for determining the total solids is to evaporate 10 c.c. in a porcelain dish or watch-glass, and dry in a water-oven to constant weight. The difference between the weight of the dish and of the dish with the solids, will give the weight of the solids in 10 c.c. of urine. Even by this method there is some loss during evaporation.

Reaction. — Normal urine is faintly acid, and grows more acid for a few hours after being voided, due to the so-called "**acid fermentation.**" During this period of acid fermentation there is frequently deposited a whitish or pinkish, or, at times, reddish sediment, due to the separation of the acid urate of sodium or to crystals of uric acid. This sediment disappears again on warming the solution. On standing still longer exposed to the air, the acidity grows less and less, and at the same time an odor of ammonia begins to be developed, and finally the reaction changes from acid to neutral, and from neutral to alkaline, with a strong odor of ammonia and more or less odor of putridity. The rapidity with which these changes take place is dependent upon the composition of the secretion, and upon the temperature, taking place more rapidly in warm than in cold temperatures. An abundance of mucus, which can usually be seen after a few hours as a light, flocculent cloud, settling near the bottom of the vessel containing the fluid, greatly hastens these fermentative changes. This is especially the case if the bladder or the kidneys are in a diseased condition. There is produced with the mucus, especially in diseased conditions of the bladder, a peculiar soluble ferment, which hastens the decomposition of urea and the production of ammonium carbonate.

The reaction of urine is best tested by dropping a small piece of a red and a blue litmus paper into the solution. If both are found red after a few minutes, the reaction is acid. If both are blue, it is alkaline. If they remain unchanged, the reaction is said to be "amphoteric." If the alkalinity be due to ammonium carbonate, the red paper, on drying and warming over a flame, turns red again. If due to the fixed alkalies, it remains blue on drying and warming.

The fermentation of urine is due to certain micro-organisms, of which the **micrococcus ureæ** is the best known. Normal urine is free from these organisms when passed, but in certain abnormal conditions it may undergo an alkaline fermentation while still in the bladder, and that apparently without the intervention of these organisms. It has been found that the fermentation may be complete in the presence of an amount of carbolic acid which is fatal to the development of micro-organisms. It has been assumed that an enzyme is secreted with the thick, mucoid secretion of vesical catarrh, which possesses active hydrolytic powers on a solution of urea. As the urine becomes alkaline, from the production of ammonium carbonate from urea, it becomes turbid and acquires a paler color. $CO(NH)_2 + 2H_2O = (NH_4)_2CO_3$. The phosphate of calcium and the ammonium-magnesium phosphate, which separate when the urine becomes alkaline, and to which the turbidity is due, are generally called the **earthy phosphates**. The latter of the two is called the triple phosphate, and is found in nearly all alkaline urines.

The acidity of the urine diminishes slightly after a full meal. It follows very nearly the acidity of the stomach contents. It is increased by gastric fermentation and in hyperchlorhydria.

The acidity of the urine increases with the amount of uric acid excreted. The **total acidity of normal urine** in twenty-four hours is equivalent to the acidity of 2 to 4 gm. of oxalic acid. This acidity is due to the acid sodium phosphate produced by the reaction of uric acid upon Na_3PO_4;

$$Na_3PO_4 + 2C_5H_2N_4O_3H_2 = 2NaHC_5H_2N_4O_3 + NaH_2PO_4.$$

Estimation of Acidity. — The acidity of the urine is largely, though not entirely, due to dihydrogen sodium phosphate, NaH_2PO_4. There is also present in the urine Na_2HPO_4 and the phosphates of calcium and magnesium. It has been shown that the presence of the calcium phosphate interferes with the direct titration of the acidity with NaOH solution and phenol-phthaleïn as the indicator. By adding to 25 c.c. of urine 15 to 20 grams of potassium oxalate, shaking for one minute and titrating with $\frac{N}{10}$NaOH, fairly correct results are obtained.

Instead of this method, it is best to estimate the NaH_2PO_4 which gives, at once, the acidity.

The method is as follows: The total phosphoric anhydride, P_2O_5, in 50 c.c. of the mixed 24 hours' urine is estimated with uranyl nitrate by direct titration, with cochineal tincture as an indicator, as described under phosphates, p. 58. The result multiplied by two will give the amount in 100 c.c.

In a second portion of 50 c.c. the disodium phosphate, Na_2HPO_4,

is precipitated with a normal solution of $BaCl_2$ (122 grams of the salt to 1,000 c.c. of water) adding about 10 c.c. of the solution for each 0.100 grms. of total P_2O_5 found by the titration.

After the addition of the $BaCl_2$, the mixture is made up to 100 c.c. in a graduated flask, filtered clear, and the phosphoric acid estimated in 50 c.c. of the clear filtrate, representing 25 c.c. of urine. The results must of course, be multiplied by four to obtain the amount of P_2O_5 existing in the form of acid sodium phosphate in 100 c.c. of the urine.

By deducting the amount of P_2O_5 thus found from the total P_2O_5, we obtain the amount of P_2O_5 in the form of Na_2HPO_4. This estimation is not quite exact, because the $BaCl_2$ precipitates not only the Na_2HPO_4, but also a small quantity of the NaH_2PO_4. It also converts a portion of the Na_2HPO_4 into normal barium phosphate, $Ba_3(PO_4)_2$ with the formation of a corresponding quantity of NaH_2PO_4 which remains in solution. It has been found by Freund and Lieblein that in urine this error is three per cent. of the amount of the P_2O_5 in the form of Na_2HPO_4. That is, the results of this last mentioned P_2O_5 are 3 per cent. too high, and this amount is to be deducted from the results found and added to the P_2O_5 existing as NaH_2PO_4.

Clinical Variations. — The urine occasionally becomes neutral or alkaline for a short time after meals, or after cold baths.

The acidity of the urine is increased:

1. In concentrated urines.
2. After prolonged exercise.
3. After meat diet.
4. Acute fevers; rheumatism, scarlatina, etc.
5. Gout, diabetes, scurvy, acid dyspepsia, usually associated with deficient oxidation of nitrogenous waste products.

The acidity is diminished or the urine is alkaline:

1. After a purely vegetable diet.
2. After taking alkaline salts, or alkaline waters.
3. Anemia, simple and pernicious, and hypochlorhydria, chlorosis, tuberculosis.
4. In certain nervous disorders. Usually associated with increase of phosphates.
5. Alkaline fermentation; cystitis.

Odor. — The odor of normal urine has been described as aromatic. A putrid odor is due to the products of decomposition. Occasionally the urine is putrid when passed, the putridity being due to the decomposition of pus, albumin, or some other foreign matter mixed with the urine in the bladder. Sulphuretted hydrogen sometimes occurs

in the urine, and a fecal odor is occasionally met with, indicating a fistulous opening between the bladder and the intestine, or an abscess between the bladder and rectum. A number of substances, when taken internally, cause the urine to assume a characteristic odor. Many aromatic substances impart their odor, as oil of turpentine (giving the odor of violets), cubebs, copaiba, asparagus, garlic, valerian, etc.

INORGANIC CONSTITUENTS OF THE URINE.

The urine contains certain inorganic salts, especially the chlorides of potassium and sodium, the phosphates of potassium, sodium, magnesium, and calcium, and the sulphates of some of these metals, and several salts of aromatic, ethereal sulphuric acid. These salts are generally tested for by the detection of the corresponding acidulous ions.

The Chlorides — Detection. — For the detection of the chlorides, add a few drops of nitric acid, and then a solution of silver nitrate (1 : 20). If the urine contains albumin, this should first be removed by boiling, adding a few drops of acetic or nitric acids and filtering. The chloride of silver separates as a white, curdy precipitate, which should occupy not more than one fourth the volume of the urine taken. If the settled precipitate occupies much more or less than one fourth the volume of the quantity of urine taken, the quantity is increased or diminished. It is always best, in making this test, to compare the specimen under examination with normal urine. In most cases this approximate estimation of the chlorides will be all that the clinician will demand. Occasionally, however, it becomes necessary to make a more accurate determination. For this purpose it is necessary to have a decinormal solution of silver nitrate — *i. e.*, a solution containing 16.96 gm. of pure silver nitrate, dissolved in a liter of distilled water.

Estimation of Chlorides. — Dilute 10 c.c. of the urine with about 90 c.c. of water, and add a few drops of a rather strong solution of potassium chromate. Now drop the silver solution from a graduated burette (see Fig. 3) drop by drop, until a permanent orange color indicates that the chlorine has all been precipitated, and that the silver has begun to form silver chromate. Ten c.c. of urine usually requires 15 to 20 c.c. $AgNO_3$ solution. One c.c. of silver solution represents 0.00354 grm. of chlorine, or 0.00584 grm. of NaCl. The results obtained by this direct titration method, while not strictly accurate, are sufficiently so for most clinical purposes. In highly colored urines this method is sometimes inapplicable, owing to the change of color being masked by the color of the urine. In such cases it is best to use the following method: Ten c.c. of the urine are mixed with 10 c.c. of a 0.5 per cent. solution of potassium permanganate, the mixture is evaporated to dryness, preferably in a platinum basin, ignited over the flame, and the chlorine estimated in the solution of the ash, as above described. Care must be taken to thoroughly exhaust the ash with boiling water before proceeding with the titration. The results obtained are good.

Second Method. — When to a solution of silver nitrate, acidulated with nitric acid, sulphocyanate of ammonium or potassium is added, a white precipitate forms, which is insoluble in nitric acid. If the fluid contains a ferric salt, a blood-red color forms at the moment when the last of the silver is precipitated. **Volhard's** method of estimating the chlorides makes use of this principle. The following solutions are needed in the process: (1) Pure nitric acid. (2) A strong solution of ferric alum (sulphate of iron and ammonia) free from chlorine. (3) A decinormal nitrate of silver solution, made by dissolving 16.96 grm. in a liter of distilled water. (4) A decinormal solution of potassium sulphocyanate, or of ammonium sulphocyanate; this should be of exactly the same strength as the silver solution; it is made by dissolving 6.5 or 7 grm. of the sulphocyanate in about 400 c.c. of water. To standardize the sulphocyanate solution, a portion of it is put into a burette, and 10 c.c. of the decinormal silver nitrate solution brought into a beaker with a few drops of the solution of iron alum. The mixture is well stirred and the sulphocyanate solution added, drop by drop, until a slight but permanent pink color appears. In accordance with the result obtained, the sulphocyanate solution is diluted to such a point that 10 c.c. of it will just neutralize 10 c.c. of the $AgNO_3$ solution. If, for example, 8 c.c. of the sulphocyanate produce a red color, we then know the amount of sulphocyanate in 8 c.c. is that which should be in 10 c.c. Therefore, we dilute the 8 c.c. with a sufficient amount of water to make it 10 c.c., or if we have 450 c.c. we shall add 2 c.c. of water, to every 8 c.c., or we make the calculation by the following proportion:

$8 : 10 :: 450 : x = 562.5$, or 450 c.c. of the solution first made up will require to be diluted to 562.5 c.c., or, there must be added 125.5 c.c. of water. Having thus corrected this solution to make it agree in strength with that of the silver solution, we again compare them to see if it is correct.

The process is conducted as follows: 10 c.c. of the urine are measured out with a pipette and placed in a graduated flask of 100 c.c. capacity; 50 c.c. of water are added, and then, successively, 4 c.c. of nitric acid and 25 c.c. of silver solution. The flask is closed with a glass stopper, and agitated until the precipitate ceases to form, and the fluid tends to clear. Distilled water is added, to the 100 c.c. mark. A portion of the fluid is then passed through a dry filter, and to 50 c.c. of this filtrate add a few drops of the iron-alum solution, and then the sulphocyanate solution from the burette until a red color appears. The amount of sulphocyanate solution added, deducted from the 12.5 c.c. of silver solution added to the 50 c.c. taken, gives the amount of silver solution used up by the chlorine in one half of the 10 c.c. of urine, or 5 c.c.

The calculation is the same as in the first method.

The Phosphates. — About two thirds of the phosphoric acid which exists in urine is combined with the alkaline metals and the remainder with lime and magnesium. These phosphates are, therefore, generally distinguished by the terms **alkaline** and **earthy phosphates.**

The **acidity** of the urine is generally believed to be due to the acid sodium phosphate, NaH_2PO_4. Sodium phosphate, Na_2HPO_4, is neutral in reaction, and Na_3PO_4 is alkaline.

Acid urines contain NaH_2PO_4, Na_2HPO_4, $CaHPO_4$, $CaH_4(PO_4)_2$, and $MgHPO_4$; while in alkaline urines we find in solution Na_3PO_4, and as precipitates $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$, and $MgNH_4PO_4$.

Detection of the Earthy Phosphates.—By adding an alkali to normal urine the phosphates of calcium and magnesium, termed **earthy**

phosphates, are precipitated. When NH_4OH is added, all the magnesium present is precipitated as NH_4MgPO_4. The phosphates of sodium and potassium remain in solution. The earthy phosphates may be approximately **estimated by adding a few drops of ammonium hydroxide solution** to the urine, and observing the amount of turbidity produced after boiling. This may be quickly done by the use of the centrifuge, in the graduated tube, and measuring the volume of the precipitate. By comparing this with the amount obtained by the same treatment of normal urine, it will indicate whether the quantity is excessive or deficient. **To detect the alkaline phosphates** precipitate the earthy phosphates with NH_4OH in slight excess, warm gently, filter, and to the filtrate add first one-half volume of NH_4Cl, and then a small quantity of $MgSO_4$ solution. The phosphoric acid in combination with sodium and potassium is thus thrown down as NH_4MgPO_4. This precipitate should be about twice as voluminous as that produced by the earthy phosphates, and whether in excess, normal, or deficient, may best be determined by comparison with normal urine.

Estimation of the Phosphates.— The phosphoric acid in urine is best estimated, for clinical purposes, by titration with a solution of uranium nitrate, using tincture of cochineal as the indicator.

Four solutions are required.

1. **Solution of Sodium Phosphate.** — Exactly 10.085 grms. of pure, non-efflorescent crystals of sodium phosphate, $Na_2HPO_4\ 12H_2O$, are weighed off and dissolved in 1000 c.c. of distilled water. 50 c.c. of this solution contains 0.100 grm. of P_2O_5.

2. **Acid Solution of Sodium Acetate.** — Dissolve 50 grms. of sodium acetate in 400 c.c. of water, and add 50 c.c. of 30 per cent. acetic acid and make up to 500 c.c. with water.

3. **Cochineal Tincture.** — About 3 grms. of powdered cochineal is digested at ordinary temperature with 100 c.c. of a mixture of 3 parts of water and 1 part of alcohol. The solution is filtered and is ready for use.

4. Prepare a solution of **uranium nitrate,** of such strength that 20 c.c. will exactly balance 50 c.c. of the sodium phosphate solution. About 40 grms. of uranium nitrate are weighed off and dissolved in a liter of distilled water. 50 c.c. of the above Na_2HPO_4 solution are measured into a beaker, 5 c.c. of solution No. 2 added, and a few drops of the cochineal tincture. The solution is now heated to boiling, and the uranium nitrate solution is run into it from a burette until a trace of permanent green color is produced. A duplicate test is always made to determine this point with as much accuracy as possible. The remaining solution should now be diluted until 20 c.c. of the uranium solution will equal 50 c.c. of the Na_2HPO_4 solution.

Example: Suppose it be found by the titration that 18 c.c. uranium solution equals 50 c.c. of the phosphate solution.

Then, for every 18 c.c. 2 c.c. of water is to be added. Or, for any desired number, say 900 c.c. of uranium solution, we may make the following proportion:

$$18 : 20 :: 900 : x. \qquad x = 1000.$$

That is, the 900 c.c. of the solution is to be diluted with water until it measures 1000 c.c. or 100 c.c. of water added. The uranium solution is not absolutely stable on long keeping, and should be restandardized every few months.

The Process: To 50 c.c. of the filtered urine add 5 c.c. of the sodium acetate solution, a few drops of cochineal solution, heat to boiling and titrate with the uranium solution until a permanent green tint is obtained.

As each cubic centimeter of uranium solution precipitates 0.005 grm. of P_2O_5, we may calculate the amount in the 50 c.c. of urine taken by multiplying the number of c.c. used by this factor.

Example: Suppose that 50 c.c. of the urine taken has required 14 c.c. of uranium solution. Then, $0.005 \times 14 = 0.070$ grms. of P_2O_5 in 50 c.c. of urine, or 0.140 grms. in 100 c.c. The quantity of P_2O_5 excreted in health is from 2 to 3 grms. per day.

As both phosphoric acid and nitrogen are derived in the body from nitrogenous tissue, we might expect a fairly constant ratio existing between these two. Such ratio has been studied and it has been found that the average man excretes by the urine 16 to 20 grms. of nitrogen per day, and from 2 to 3 grms. of P_2O_5. This would give a ratio of about 1 to 5 or 6.

The normal ratio of P_2O_5 to urea is about 1 to 10.

Sulphates. — Sulphuric acid occurs in the urine partly in combination with the metals, and partly in combination with certain aromatic bodies of putrefactive origin, called collectively the ethereal sulphates. The most important of these are phenol- and cresol-potassium sulphates, indoxyl- and skatoxyl-potassium sulphates, pyrocatechin- and hydrochinon-potassium sulphates. The two classes of sulphates are generally distinguished as the **preformed** or **mineral sulphates** and the **conjugate** or **ethereal sulphates.** About nine tenths of the total sulphuric acid is combined with the metals or is preformed. About one tenth exists as ethereal sulphates of potassium.

Detection. — The preformed H_2SO_4 is detected by the addition of $BaCl_2$ in the presence of free HCl. It precipitates as a fine, white precipitate of $BaSO_4$, rendering the solution opaque, and milk-like in appearance. An approximate estimate may be made by comparing the turbidity with that of normal urine treated in the same way. An excess of sulphuric acid may be due to the taking of an excessive amount of sulphates with food or drink.

Detection of the Ethereal Sulphates. — The ethereal sulphuric acid is not precipitated by barium chloride. It is necessary to break up these compounds by boiling with a strong acid like nitric or hydrochloric acid before applying the usual test. They are thus broken up into free sulphuric acid and the aromatic substance. To test for them a slight excess of barium chloride and a few drops of acetic acid are added and the $BaSO_4$ filtered off. It may be necessary to pass the solution through the filter a number of times to obtain a perfectly clear filtrate. The clear filtrate is now mixed with about one fifth its volume of HCl and boiled and then heated on a water-bath

for a half hour. The $BaSO_4$ thus obtained represents the sulphuric acid existing as ethereal sulphates.

In most cases it is more satisfactory to test for the indoxyl, than for the sulphuric acid in these conjugate sulphates, as hereafter described.

Estimation of the Preformed Sulphates.—This may be done by the gravimetric or volumetric method. The former is done by precipitating the SO_4 as $BaSo_4$ with $BaCl_2$ in presence of free HCl. The precipitate is collected on a filter, washed, dried, ignited and weighed, and the acid calculated from the weight of the $BaSO_4$. While this process gives accurate results, it consumes too much time to be available for clinical purposes.

The volumetric methods are less accurate but are sufficiently so for most clinical purposes. Freund's method is to precipitate the preformed sulphuric acid with a standard solution of barium acetate, using alizarin-mono-sulphonate of sodium as an indicator. To 50 c.c. of the urine are added 10 drops of the alizarin solution, then a 5 per cent. solution of acetic acid, until the last trace of red color is discharged, and then 5 c.c. more. The solution is now heated on a water-bath to near the boiling point, and the barium acetate solution, containing 9.579 grms. per liter, is run in until a distinct red color again appears. 1 c.c. of the barium solution precipitates 0.003 grm. of SO_3.

The **conjugate or ethereal sulphates,** are estimated by a second titration in 50 c.c. of the urine, which has been heated to boiling with one tenth volume of HCl. The dark color thus produced is bleached by the addition of a small piece of zinc, and heating for a few minutes longer. The HCl is neutralized and the zinc precipitated by sodium carbonate, the solution filtered, and treated as above.

This gives the total sulphates, the difference between the preformed and total gives the ethereal sulphuric acid.

Clinical Variations of the Inorganic Salts. — The most important inorganic constituents of urine, from a diagnostic point of view, are the chlorides, phosphates, and sulphates. We may include among these, for convenience, calcium oxalate, although the acid is organic.

CHLORIDES. — Daily amount, ten to fifteen grms. (150 to 200 grs.).

Increased: 1. After muscular exercise.
2. After taking much food or drink.
3. During absorption of exudates.
4. In acute Bright's disease, diabetes insipidus, and ague, after chill.

Diminished: 1. In all acute fevers, especially acute pneumonia. (Increases after crisis.)
2. During formation of exudates and dropsies.
3. In chronic nephritis, certain dyspepsias, diarrheas, cholera, typhoid.

PHOSPHATES. — Daily amount of phosphoric acid, two to four grms. (30 to 60 grs.). The ratio of P_2O_5 to urea is fairly constant in health at 1 to 10.

Increased: 1. After mental work, hysteria, certain nervous diseases, or worry.
2. After muscular exertion, meat diet, copious drinking of fluids.
3. Rachitis, osteomalacia, early phthisis, diabetes mellitus, phosphatic diabetes, some fevers.

Diminished: 1. Most acute diseases, renal diseases, rheumatism, gout, pregnancy.

SULPHATES. — Daily amount of sulphuric acid, 1.5 to 3 grms. (22 to 45 grs.). (See page 59.)

Sulphuric acid occurs as preformed and as ethereal sulphates. Little diagnostic value is attached to variations in the preformed sulphates.

Increased: 1. Meat diet, prolonged exercise, after taking sulphur compounds.
2. Fevers, rheumatism, pneumonia, delirium tremens, cerebral meningitis.

Diminished: 1. Vegetable diet (usually).
2. Nephritis, chlorosis, chronic diseases generally.

Ethereal Sulphates are Increased: After a vegetable diet, and from putrefaction of proteids in the intestines.

ORGANIC CONSTITUENTS.

Carbamid or Urea. — $CO(NH_2)_2$. Urea is the most important constituent of the urine, as it is the chief condition in which nitrogen leaves the body.

As about 85 to 88 per cent. of the nitrogen from tissue metabolism escapes from the body in the form of urea, we may regard the urea as a measure of the tissue changes, provided, of course that the kidneys are able to eliminate what is produced. When the kidneys are diseased there is usually a diminished amount of urea eliminated.

The quantity of urea is a matter of great importance in the study of metabolism, and in the study of renal diseases.

Detection. — Urea may be detected by evaporating a few drops of urine on a glass slide, moistening the residue with nitric acid, or a strong solution of oxalic acid, and allowing it to crystallize, and examining the crystals of urea nitrate, $CO(NH_2)_2HNO_3$, or oxalate $2CO(NH_2)_2.H_2C_2O_4.H_2O$, under a microscope of low power.

On boiling a solution of urea with strong alkalies, it is converted into ammonium carbonate, which then evolves ammonia gas, NH_3, as represented by the following equations:

$$CO(NH_2)_2 + 2H_2O = CO_3(NH_4)_2$$
$$(NH_4)_2CO_3 + 2NaOH = 2NH_3 + Na_2CO_3 + 2H_2O.$$

Estimation. — The amount of urea in urine is generally looked upon as an index of the nitrogenous metabolism going on in the body, or of the eliminating power of the kidneys. The quantity of urea excreted in twenty-four hours by a healthy adult of 150 pounds body-weight, and doing ordinary work, is usually stated to be from 30 to 33 grms., or from 430 to 550 grs. The quantity will be increased by an increased consumption of nitrogenous food or by hard work, and it is diminished by a non-nitrogenous diet and by little exercise. Roughly, in the absence of sugar, albumin, and other abnormal ingredients, one half the total solids may be regarded as urea. The more accurate quantitative estimation requires so little time, apparatus, and skill that it is now very generally employed. The determination is based upon the fact that urea is decomposed by alkaline hypochlorites or hypobromites into carbon dioxide, water, and nitrogen.

$$CO(NH_2)_2 + 3NaBrO = 3NaBr + CO_2 + 2H_2O + N_2.$$

If the solution of hypobromite contains an excess of NaOH or Na_2CO_3, the CO_2 enters into combination with these and only the nitrogen escapes, and the amount is determined by measure.

One c.c. of nitrogen at the ordinary temperature and pressure corresponds to about 0.0027 grms. of urea.

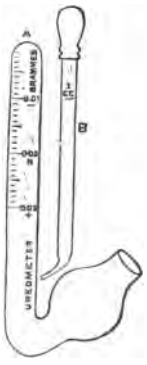


FIG. 6.

The **hypobromite solution** is prepared as follows: 100 grm. of NaOH are dissolved in 250 c.c. of water, and to this solution, when cold, 25 c.c. of bromin are slowly added, and the solution kept cold. This solution contains sodium hypobromite, hydroxid and bromate. The solution should be freshly prepared, as it readily undergoes decomposition. Owing to the instability of this solution and the excessively disagreeble handling of bromine, the author employs a solution of sodium hypochlorite, or chlorinated soda, with the addition of KBr. This solution acts as well, by the method to be described, as the above. Various forms of apparatus have been devised for the quantitative estimation of urea. One of the simplest of these is that devised by Dr. C. A. Doremus, which is represented by Fig. 6.

The Process. — The tube, A, is filled with the above-mentioned solution of hypobromite, and 1 c.c. of urine is introduced with the pipette, B, as nearly as possible at the center of the lower portion of the upright limb. The urea is decomposed and the N rises to the upper, closed end. After the decomposition is complete, which requires from 15 to 30 minutes, the urea is determined by reading the graduations at the surface of the column of liquid. This ureometer, according to the graduation, gives

either the milligrams of urea in 1 c.c. of urine, the percentage, or grains per fluidounce.

The Author's Method. — The author uses a graduated tube, about one centimeter in diameter and 50 cm. long, closed at one end. The graduations indicate at once the number of grains of urea in a fluidounce of urine, when 1 c.c. is taken for the estimation. (See Fig. 7.) The ordinary **gas-tube** may be used, when the readings will give the c.c. of nitrogen, from which the urea is calculated, by multiplying the number of c.c. of nitrogen by the factor 0.0027.

The process is conducted as follows: A 20 per cent. solution of KBr is added to the fifth division of the ureometer. Chlorinated soda solution, of good quality, is then added to the fifteenth or twentieth division. The author would advise the use of Squibb's assayed product, or some other equally good solution. The tube is now inclined and pure water poured carefully down the side of the tube and floated upon the top of the fluids already in it; 1 c.c. of urine is then added, in the same inclined position, so that it will not mix with the reagents below, but remain in the water at the surface of the fluid. The tube is now firmly grasped in the right hand, with the ball of the thumb tightly pressed upon the open end. The tube is now inverted a few times so as to thoroughly mix the contents of the tube. A rapid decomposition takes place, which is usually ended in from three to five minutes. During this time the liquid is kept agitated without violent shaking. As soon as the effervescence has ceased, the reading is taken at the surface of the fluid, with the tube held in the inverted position. It is now opened under water, when the column of fluid in the tube will fall, and the reading is again taken. It is best to have a wide, deep jar for the water, so that the tube may be depressed to bring the surface of the liquid in the tube to the surface of the water in the jar; but an ordinary bowl may be used, as the error caused by the difference in level of three or four inches of water is very slight. The difference in the two readings gives the number of grains of urea in a fluidounce of urine. This quantity, multiplied by the number of fluidounces passed in twenty-four hours, gives the amount of urea excreted in twenty-four hours, which should be from 400 to 500 grains. **A less quantity of urea than 350 grains in an adult of 150 pounds body-weight, who is eating the usual amount, should be regarded as pathological, and suspicious of renal insufficiency, or nephritis.**

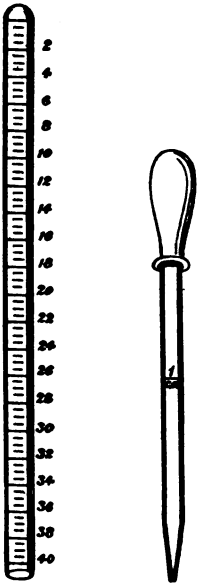


FIG. 7. — UREOMETER.

Clinical Significance. — We may look upon the amount of urea eliminated as a measure of tissue change. This is only true in health. In certain diseases — as, for example, in yellow atrophy of the liver—the urea may almost disappear from the urine, and the nitrogen will escape in some other form. When we wish to measure the tissue metabolism, we usually determine the total nitrogen eliminated by the kidneys. It is usual to express the nitrogen as urea. One hundred grms. of muscle contain about 3.4 grms. of nitrogen, corresponding to 7.286 grms. of urea. Or, 1 grm. of urea is equivalent to 13.72 grms. of muscle tissue, and 1 grm. of nitrogen is equivalent to 29.4 grms.

of muscle tissue. If we know the amount of nitrogen taken as food, and the amount excreted by the urine and by the feces, we can calculate just what is going on in the body. When the amount of intake and excretion of nitrogen are equal, the body is said to be in nitrogenous equilibrium — *i. e.*, it is neither gaining nor losing nitrogen. As the amount of urea must vary with the amount of nitrogen ingested, a mere statement of the amount of urea excreted in twenty-four hours, without taking into account the amount of nitrogen taken as food, can not mean very much. The nitrogenous metabolism must be more in a large, robust man than in a feeble half-fed one. It must be more in a man of 200 pounds than in one weighing 125 pounds. We may formulate this fact in this manner: For every 100 pounds in body-weight, an adult ought to excrete about 20 to 25 grms. of urea in each twenty-four hours. Children excrete more in proportion to their body-weight than adults. Thus, from three to six years of age, they secrete for every pound of body-weight 0.45 grms. of urea in each twenty-four hours; at eight to eleven years, 0.36 grms.; at thirteen to sixteen years, from 0.18 to 0.27 grms.

The excretion of urea is increased in all acute fevers. In such cases the patient eats little, and excretes more urea and less chlorides than in health. The ratio between urea and chlorides may sometimes assist us in diagnosis of certain obscure wasting diseases. In acute febrile diseases the excretion of urea has the following course: At the beginning and until the acme is reached, urea is increased to 40, 50, or even 60 grms. in twenty-four hours. It then grows less, and falls below normal as the fever abates, and gradually comes up to normal again during convalescence. In intermittent fever the urea is increased before the chill or paroxysm.

In those febrile diseases which end by crisis, as pneumonia, the increased elimination of urea may continue for several days after the crisis. Another exception to the general course of the elimination of urea, is in the disease known as acute yellow atrophy of the liver. In this disease the urea nearly or entirely disappears from the urine, and is replaced by leucin and tyrosin. In most chronic diseases, with diminished nutrition, it is below normal. In hectic exacerbations it increases during the fever. Near the fatal termination of fevers the urea decreases very much, or, it may almost disappear during the last twenty-four hours. Among the non-febrile diseases in which urea is increased, the most marked case is diabetes mellitus, in which it often rises as high as 100 to 150 grms. in twenty-four hours. A partial explanation of this large amount of urea is found in the large amount of food taken by such patients, although there is an increased tissue destruction in this disease. A moderate increase has been noticed in cases of pernicious anemia, severe cases of leucocythemia, scurvy, chorea and paralysis agitans. There is usually an increase after epileptic seizures, and some have noticed the same thing after attacks of hystero-epilepsy. **Certain drugs cause an increased elimination of urea.** An increase is produced by coffee, caffeine, morphine, ammonium chloride, NaCl, KCl, Li_2CO_3, and the ingestion of a large quantity of water. In regard to the effects of quinine, salicylic acid, and cold baths, opinions differ. It is greatly increased in phosphorus poisoning. Electrical stimulation seems to increase the elimination of urea. **The elimination of urea is decreased** by any cause that interferes with hepatic activity, as in all organic diseases of this organ. It is very much decreased in carcinoma and cirrhosis of the liver, and in acute febrile jaundice (Weil's disease). A careful estimation of the output of urea is then often of service in the diagnosis of hepatic disease. In chronic lead poisoning urea is decreased. Also in Addison's disease. In most forms of nephritis, especially in all forms in which the tubules of the kidneys are affected, urea is diminished. As urea is separated by the epithelium lining of the convoluted tubes, any disease of this epithelium will interfere with its excretion.

The Ratio between the Excretion of Urea and Chlorides. — In normal conditions the chlorides, calculated as NaCl, are about one half as much as the urea. When the urea is being derived from the destruction of tissue, or when the body is living upon its own proteids, this ratio will be changed, for the tissues contain much less NaCl than our average food. In total abstinence from food, the NaCl almost disappears from the urine. In all febrile diseases, we should expect that this ratio would be disturbed, as it usually is.

Uric acid, $C_5H_4N_4O_3$, is a constituent of normal urine, sometimes occurring in the free state, but oftener in combination with potassium, sodium, ammonium, and occasionally with calcium and magnesium, usually called the **urates.**

Uric acid is soluble in 14,000 parts of cold water, and is, therefore, frequently met with as a sediment, and is then detected by microscopical examination. It varies in quantity from 0.4 to 1.0 grm. (from 6 to 15 grs.) in twenty-four hours. The normal ratio of uric acid to urea is about 1 : 40.

Detection. — It is best recognized, when in the free state, by the microscope. The crystals, as seen with this instrument, are colored yellow or reddish by the pigment uroerythrin, and appear in a variety of shapes, the most common being the "lozenge" or "whetstone" shape.

Chemical Tests. — No. 1, Murexid Test.—Evaporate a portion of the urine to dryness in a porcelain dish upon a water-bath. Moisten the residue with nitric acid, and, after evaporating off the acid, moisten the residue with ammonium hydroxide. If uric acid be present, either in the free or combined state, the residue assumes a beautiful purple-red color, due to the formation of murexid $C_4H_5N_3O_3$. The reaction is said to occur also with xanthin, hypoxanthin, tyrosin, and some other bodies.

No. 2, Carbonate of Silver Test. — Render the urine decidedly alkaline with Na_2CO_3 or K_2CO_3, and moisten a filter-paper with the liquid. Now touch the moistened paper with a glass rod dipped in a solution of $AgNO_3$. A distinct gray stain indicates the presence of uric acid.

Estimation of Uric Acid. — The estimation of uric acid is usually attended with much difficulty and consumption of time. The author has devised the following volumetric method, which is fairly rapid, reasonably accurate, and requires little skill. It can therefore claim a place as a clinical method. The process is based upon the well-known fact that uric acid is completely precipitated from its solutions, containing an excess of ammonium-magnesium mixture and ammonium

hydroxide, by silver nitrate. When the precipitation is complete, the slightest trace of silver in solution is shown by the dark color produced, in a drop of the clear solution, by a soluble sulphide.

The solutions required are:

1. **A $\frac{1}{50}$ normal solution of $AgNO_3$**, made by diluting 1 volume of a $\frac{N}{10}$ solution with 4 volumes of distilled water.

2. **Magnesium mixture,** made to contain about 10 grms. of crystallized $MgSO_4$, 12 grms. of NH_4Cl, and 100 c.c. of aqua ammoniæ (U. S. P.).

3. A solution of **ammonium sulphydrate,** or **sodium sulphide.** A solution of sodium sulphide may be extemporized by boiling 5 c.c. of the ordinary solution of NaOH with a small quantity of flowers of sulphur until the solution is of a deep yellow color, and diluting. This solution should be freshly made and of such strength that its color is nearly that of the urine.

When the urine contains a sediment of uric acid, or acid urates, it is to be put in solution by warming with a few drops of NaOH solution, before beginning the process, and the excess of alkali neutralized with acetic acid. In very dark fever-urines it is best to dilute with an equal volume of water. The titration is performed in a hot solution, to prevent the precipitation of the xanthin bases by silver nitrate.

The process is conducted as follows: To 50 c.c. of the clear urine add 5 c.c. of the magnesium mixture and about 10 c.c. of ammonium hydroxid (sp. gr., 0.960), or enough to give a decided excess. Warm the solution on a water-bath, and add from a burette a $\frac{1}{50}$ normal solution of silver nitrate. From time to time a drop is removed from the solution, by means of a dropper-pipette, with a bit of absorbent cotton wound tightly over the end so as to make an efficient filter, and, after removing the cotton filter, bring a drop of the solution in contact with a drop of the weak sodium sulphide solution on a white porcelain surface. Experiments with pure water shows that it requires ½ c.c. of the silver solution in 50 c.c. to give a marked reaction. This amount must therefore be deducted from the reading. The titration is continued until a dark ring or cloud is seen at the line of contact of the two drops, showing the presence of silver in the solution. Each c.c. of silver solution corresponds to 0.00336 grms. of uric acid, and the number of c.c. used (less ½ c c. for each 50 c.c. of urine), multiplied by this factor, gives the number of grams of uric acid in the urine taken. From this we may easily calculate the amount in 100 c.c. or that excreted in twenty-four hours.

As soon as the process is complete, the precipitate rapidly settles, and it is best to draw off a drop or two from this clear supernatant liquid and test it carefully again. We may also check our work by running in another drop of the silver solution, to see if it produces a cloud, or to see if the precipitation be complete. As there is no excess of silver in the hot liquid at any time, there can be no reduction of silver.

If, after the titration is complete, the solution be cooled, it will usually be found that it will require from 1 to 3 c.c. of the silver solution to again produce the end reaction, because of the precipitation of the purin bases as silver compounds. The formula of the xanthin-silver compound is $Ag_2O.C_5H_4N_4O_2$. The factor for the $N/50$ $AgNO_3$ solution is 0.0015 — that is, if we calculate them all as xanthin, each c.c. of silver solution used in the cold solution, more than is required by the hot solution, corresponds to the above amount of purin bases.

By making two titrations, the one in the hot and the other in the cold urine, we may estimate both the uric acid and the purin bases, the latter by the difference in the results of the two titrations.

Ruemann's Iodometric Method. — This method is based on the assumption that uric acid is the only constituent of the urine that will absorb iodine at the ordinary

temperature. This assumption has been criticized, but the method is easy to work, and the results obtained in the author's laboratory agree fairly well with the above method and with known added amounts of uric acid. The apparatus devised by Ruemann consists of a graduated tube (*uricometer*), closed at one end and provided with a glass stopper ground in at the other. *The reagent* consists of a solution of 1.5 grms. iodine, 1.5 grms. potassium iodide and 15 grms. absolute alcohol in 185 c.c. of water.

The process is conducted as follows: Carbon disulphide is added to the first line (marked *S* on the instrument) and the above reagent is added up to the next mark (*J* on the instrument). The iodine solution must be added by means of a pipette that will pass into the *uricometer*, and delivered so as not to soil the sides with the solution. Add the urine, which must be brought to the temperature of 18° C. (65° F.), until it reaches the lower part of the graduated scale, which is marked 2.45.

Close the open end of the uricometer with the glass stopper, grasp the tube firmly in the right hand, with the thumb holding the stopper in place, and shake vigorously. The CS_2 will assume a dark brown color. Remove the stopper and add more urine and shake vigorously for one minute. Repeat this addition of urine until the free iodine just disappears, which is shown by the CS_2 becoming a pure white color. Read off on the scale at the surface of the liquid in the tube. The graduations show the amount of uric acid in grams per liter; or, by prefixing a cipher to the readings, the per cent. Traces of albumin need not be removed, as they do not interfere, but a large amount of albumin, blood or pus should be removed by coagulation and filtration. If the urine be alkaline in reaction it must be rendered acid with dilute acetic acid before applying this process. Uric acid contains 33.3 per cent. of nitrogen, while urea contains 46.66 per cent. of nitrogen.

The Purin Bodies or Xanthin Bases. — Under the term purin bodies are included a considerable number of related bodies found in normal urine, which are believed to be derived from the nucleins of the body, and certain related substances found in our foods and taken into the system with them. In recent years much importance has been attached to their quantitative variations in the urine. These bodies are **adenin, guanin, hypoxanthin, xanthin, heteroxanthin, paraxanthin** and **uric acid.**

As all these bodies are believed to have the same origin, it is believed by many that the estimation of the total purin bodies gives more useful information to the clinician, than the estimation of the uric acid alone.

In the above description of the author's titration method for uric acid, it is pointed out that it may be employed to estimate both the uric acid and the total purin bodies, and by difference, the xanthin or purin bases. Hall has devised the following very ingenious process of estimating the total purin bodies based upon this principle.

Hall's Purinometer. — This instrument, as the name indicates, is designed to estimate the total purin bodies in foods and in the urine. It consists of an upright cylinder or tube, divided into two chambers by a large stopcock. The tube forming the upper chamber is

graduated from the stopcock upward into 100 divisions. The lower chamber is not graduated. The instrument is mounted on a wooden base. Two solutions are needed:

1. Ten grms. of magnesium chloride are dissolved in about 75 c.c. of water. 10 grms. of ammonium chloride are dissolved in the same solution. To this solution is added 100 c.c. concentrated ammonia solution. If a precipitate occurs, more ammonia is added until the solution becomes clear. Water is now added to make the volume up to 200 c.c. and 10 grms. of purified talcum added.

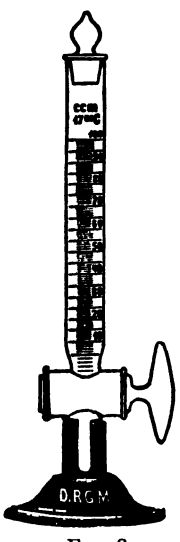


FIG. 8.

2. Silver nitrate, 1 grm.
   Strong ammonia water, 100 c.c.
   Talcum, 5 grms.
   Distilled water, 100 c.c.

The process is conducted as follows:

The total amount of urine of the 24 hours is collected and mixed. If albumin, pus or blood are present, they must be removed by boiling in slightly acid solution. Close the stopcock between the two chambers of the apparatus and pour in urine to the 90 c.c. mark, and add 20 c.c. of solution No. 1. The phosphates are at once precipitated and the tap is turned vertical with the tube. In ten minutes the phosphates will have passed into the lower portion of the tube and the tap is again turned at right angles, and solution No. 2 added up to 100 c.c. The resultant precipitate of silver-purin should be pale yellow. Incline the purinometer backwards and forwards until all the white silver chloride is dissolved. If this does not occur, add a few drops of strong ammonia, or use diluted urine. Place the apparatus in a cupboard, away from the light, and read off the number of cubic centimeters occupied by the precipitate after twenty-four hours.

The following table shows the nitrogen percentage yielded by the given volume of the precipitate. Multiply this factor by the number of hundreds of cubic centimeters contained in the 24 hours' urine.

Volume of Precipitate.	Per cent. Purin-Nitrogen.
4 c.c	0.0078
5 c.c	0.0097
6 c.c	0.0117
7 c.c	0.0136
8 c.c	0.0156
9 c.c	0.0175
10 c.c	0.0185
11 c.c	0.0195
12 c.c	0.0205
13 c.c	0.0218
14 c.c	0.0225
15 c.c	0.0234
16 c.c	0.0249

Volume of Precipitate.	Per cent. Purin-Nitrogen.
17 c.c.	0.0260
18 c.c.	0.0265
19 c.c.	0.0270
20 c.c.	0.0275
21 c.c.	0.0283
22 c.c.	0.0286
23 c.c.	0.0299
24 c.c.	0.0312
25 c.c.	0.0325

Example. — A urine gave 15 c.c. of precipitate. This contains 0.0234 grms. nitrogen. The total amount of urine passed was 1160 c.c. $.0234 \times 11.60 = 0.271$ grms. of purin nitrogen passed in 24 hours.

Clinical Significance and Variations. — The amount of uric acid excreted by a healthy man is subject to rather wide variations, ranging from 0.2 to 1 grm. in twenty-four hours. The quantity excreted in the urine is not always an exact measure of the amount produced in the system, for it has been shown that many foods contain purins which add to those produced by the tissues, and that they can be, and often are, stored up in the tissues. The formation and the elimination of uric acid are two entirely different things. Both of these factors are of the greatest importance in diagnosis and treatment. Uric acid is a dibasic acid, and hence forms neutral and acid salts. It occurs in the urine as free acid, as neutral sodium and potassium and ammonium urates, and as acid urates of sodium and probably of ammonium. The neutral salts are quite freely soluble in water, while the acid salts are soluble with difficulty, and the free acid is almost insoluble and appears as a more or less colored sediment, soluble in 14,000 parts of cold water, and in 1,800 parts of boiling water. It is soluble in alkalies, even in the carbonates of the fixed alkalies. The acid is soluble in sodium phosphates, and these salts in the blood serve to hold it in solution in that fluid. It reacts with the sodium phosphate of the blood to produce the neutral or acid urate, according as it is present in smaller or larger quantities :

$$2Na_2HPO_4 + C_5H_4N_4O_3 = 2NaH_2PO_4 + Na_2C_5H_2N_4O_3$$

or

$$Na_2HPO_4 + C_5H_4N_4O_3 = NaH_2PO_4 + NaHC_5H_2N_4O_3.$$

The greater the proportion of sodium phosphate in the blood, the greater is the solvent power of this fluid for uric acid. Hence, when we wish to increase the elimination of uric acid, with a given production of it, we do so by increasing the alkalinity of the blood. When the alkalinity of the blood runs down, it is capable of proof that the uric acid is stored in the tissues, to be dissolved out and thrown into the circulation whenever the alkalinity rises again. From these facts it is evident that a single estimation of the amount of uric acid, or of the total purin bodies, on a given day does not necessarily give the production for that day. The purin bodies, when present in excess in the circulation, are believed to give rise to certain fairly well-defined symptoms, such as headaches, muscular pains, neuralgias, nervous irritability, irritable heart with increased blood tension, gout, and nephritic irritation. Excess of uric acid excretion may be the result of an increased ingestion of purin-containing foods, an increased production of the acid from body tissue or of an increased elimination. The quantitative estimation of uric acid is time-consuming, and, as we have seen, a number of such determinations are necessary to make these determinations of much value. Persons differ very much as to their tolerance of uric acid, some being

very sensitive to a slight increase of it, while others seem to tolerate large quantities without marked symptoms. There is, in health, a fairly constant relation between the amount of uric acid and the urea excreted. The ratio, as stated by different authors, varies somewhat according to experience, and according to the methods used to estimate the urea and uric acid. Some place it as low as 1 to 50 or 60, while others at 1 to 35 or 40. Haig, who examined his urine for 2,013 days, found the ratio to be about 1 to 33 or 35. Yvon and Berlioz found it 1 to 30 in one set of experiments, and 1 to 40 in another. Lecanu found 1 to 33. Simon gives it as 1 to 50 or 60, which latter ratio is probably low. My experience with the methods given in this book makes the ratio about 1 to 40. *When studying this ratio, the patient should be put upon a purin-free diet of milk, eggs, cheese, rice, wheat bread, sugar and butter.*

About one half of the purin bodies of meats are excreted within 48 hours, as uric acid and xanthin bases. In pathological states the purins often run as high as 1 to 20, or in leucocythemia as high as 1 to 12. The purins are usually increased in most febrile diseases, as typhoid, pleurisy, pneumonia, pericarditis, rheumatism, etc. It is generally increased in gout, chlorosis, anemia and chronic nephritis.

ABNORMAL CONSTITUENTS OF URINE.

These are albumin, globulin, albumose, peptone (?), glucose, acetone, diacetic acid, bile-coloring matters, biliary acids, blood, blood-coloring matters, pus, chyle, and abnormal sediments, such as tube-casts, excessive amount of epithelium cells, mucus, etc.

Albumin. — Albumin is found in the urine, at times, without apparent disturbance of health. Usually, however, it is regarded as pathological, and is so often associated with various organic diseases of the kidney, that its presence is often taken as evidence of some one of these diseases. It occurs principally in the form of serum-albumin. It is coagulated by a temperature of from 73° to 75° C. (163.4° to 167° F.). **In all cases the urine should be clear before applying the tests for albumin.** If not clear, it should be either settled and decanted, or filtered. It is sometimes necessary to shake the urine with pulverized talcum, or other powder, before filtering, to get it clear.[1] The tests for albumin usually depend upon its coagulation and the formation of a turbidity in the solution. A few tests depend upon a change in color. The tests that are most satisfactory when applied to the urine are as follows:

1. **Heat and Nitric Acid Test.** — Heat about 5 c.c. of the urine to boiling in a test-tube. A turbidity, if present, will be due to **albumin** or **earthy phosphates.** Now add, slowly, a few drops of acetic or nitric acid. If the turbidity be due to the phosphates, it disappears; while if due to albumin, it remains permanent. Care must be taken,

[1] Turbid urines may be rendered clear by adding a few drops of $MgSO_4$ solution, then an excess of NH_4OH, and filtering from the phosphates. The urine is then acidified with acetic acid, before applying the tests.

in the addition of the acid after boiling, to note the effect after each drop is added, and to go on adding it until there can be no doubt that the urine is distinctly acid. **A large excess of acid is to be avoided.** This test will show traces of albumin only under the most favorable conditions.

2. **Heller's Test. The Contact Method.** — Place 2 c.c. of pure HNO_3 in a narrow test-tube, and, inclining the tube to one side, pour the urine carefully down the side of the tube so that it may float upon the acid. This is best done with a dropper-pipette, or by pouring the urine from one test-tube into another, holding both in a nearly horizontal position. If this be carefully done, there will be very little admixture of the two liquids. If albumin be present, a white, opaque zone of coagulated albumin appears at the line of contact of the two fluids. A brown zone will frequently be seen at this point, which grows in intensity on standing, and is due to the action of the acid on the coloring matters, but it does not give any turbidity unless albumin is present. If bile be present, the color may be green ; if blood, brown-red. This test is decidedly more delicate than No. 1.

Precautions. — Occasionally after the administration of turpentine, or balsams and resins, these are precipitated by HNO_3 as a yellow-white cloud, which, however is soluble in alcohol. I have never seen the uric acid, sometimes set free by HNO_3, resemble the precipitated albumin nearly enough to be mistaken for it.

Roberts modifies this test by using, instead of pure nitric acid, a mixture of 1 volume of HNO_3 and 5 volumes of a saturated solution of $MgSO_4$. This reagent is as sensitive as HNO_3, and pleasanter to handle. It is used in the same way.

3. **Acetic Acid and Potassium Ferrocyanide.** — Acidulate the urine with acetic acid, filter if much nucleo-albumin is precipitated, and then add a few c.c. of a solution of potassium ferrocyanide. Or, better, float the acidulated urine over the K_4FeCy_6 solution. If albumin be present, it appears as a white gelatinous precipitate. This reagent does not precipitate mucin or alkaloids ; it does precipitate albumose and nucleo-albumin. The former dissolves in excess of acetic acid. It is a very delicate and reliable test.

4. **Picric Acid with Acetic Acid.** — A cold, saturated solution of picric acid may be used by the **contact method,** after previous acidulation of the urine. At the line of contact the albumin appears as a white zone. Heat afterward to dissolve alkaloids, mucin, and urates, which are precipitated with the albumin. It is better to heat the urine before adding the reagent.

5. **Sodium Tungstate Solution. — Reagent:** Made by mixing

equal parts of cold, saturated solutions of sodium tungstate and citric acid. As its specific gravity is heavier than that of urine, it is best applied by the contact method, adding the reagent first. This is an extremely delicate test, and precipitates at the same time albumoses, mucin, some alkaloids and urates, all of which, except mucin, are dissolved by heating.

6. **Tanret's Test (Potassio-mercuric Iodide Test).** — Reagent prepared as follows: Mercuric chloride, 1.35 grm.; potassium iodide, 3.32 grms.; acetic acid, 20 c.c.; distilled water, 80 c.c. The $HgCl_2$ and KI are separately dissolved in water and then mixed, and the acetic acid afterward added. The resulting liquid is heavier than urine (sp. gr., 1040), and is best used by the contact method. It is exceedingly delicate, detecting 1 part of albumin in 20,000 parts of urine. Heat to dissolve the alkaloids, mucin, and peptone, as in tests 4 and 5.

Jolles suggests the following very delicate reagent: Mercuric chloride, 10 grms.; succinic acid, 20 grms.; sodium chloride, 10 grms.; distilled water, 500 c.c.

7. **Acidulated Brine Test. — Reagent:** To a pint of a saturated solution of common salt add 1 ounce of HCl, and filter if necessary. This is a delicate test for albumin when properly used. It has a high specific gravity, and is best used as follows: The solution is heated to boiling, and the urine added by the contact method. If albumin be present, it appears as a zone at the contact surface. It does not precipitate peptone, albumose, or the alkaloids.

8. **Trichloracetic Acid.** — This is a white, crystalline acid, sometimes employed as a test for albumin. It may be used in the form of a saturated solution, by the contact method, or the crystals may be added directly to the urine, when they will form a strong solution at the bottom of the tube. It presents no decided advantages over the tests above mentioned.

9. A solution of **salicyl-sulphonic acid** in water, or the crystalline acid added directly to the urine, is a very delicate test for all forms of proteids, precipitating albumoses and peptones, but not alkaloids or mucin. The albumoses and peptones dissolve on heating, to reappear on cooling. A large excess of mucin, or nucleo-albumin, may give a cloudiness with this reagent.

10. **Metaphosphoric** or **glacial phosphoric acid** has also been recommended by some as a reliable test for albumin in urine. A solution of **resorcinol** in water (1:3) has also been highly recommended.

Albumin test-papers, suggested by Dr. Oliver, may be prepared by saturating bibulous paper in solutions of potassio-mercuric iodide, of

potassium ferrocyanide, and of citric acid. To use these papers the urine is acidified with one of the citric acid papers, and then either a potassio-mercuric iodide or a potassium ferrocyanide paper added.

Estimation. — The **quantitative** estimation of albumin is of considerable importance, but somewhat difficult to perform. Comparative tests are all that the clinician will usually find necessary. The easiest approximate method is to boil a given quantity of urine in a test-tube, add 2 or 3 drops of nitric acid, set aside for twelve hours, and note the volume occupied by the precipitated albumin. This is generally spoken of as **volume per cent., and has no relation to actual percentage.**

FIG. 9.

A more accurate method, and one sufficiently so for clinical purposes, is with **Esbach's albuminometer.** This consists of a graduated glass tube, shown in Fig. 11. To use the instrument, fill to U with urine, and to R with the test liquid. Close the tube by a rubber stopper, mix by agitation and set aside for twenty-four hours. Each of the main divisions which the precipitate covers represents 1 grm. of albumin in 1 liter of urine.

Test solution:	Picric acid,	10 grm.
	Citric acid,	20 grm.
	Water,	1,000 grm.

Centrifugal Method. — To 10 c.c. of the filtered urine in a graduated centrifugal tube (Fig. 12, C) add 3.5 c.c. of a 10 per cent. solution of $KyFeCy_6$ and 1.5 c.c. of acetic acid, and place the thumb over the open end of the tube and mix thoroughly. Place the tube in the centrifuge and rotate at a velocity of about 1,000 per minute for three to five minutes, or until the supernatant fluid is perfectly clear. According to Ogden each $\frac{1}{10}$ c.c. of precipitate represents $\frac{1}{60}$ of 1 per cent. of albumin by weight.

Serum-globulin, or **paraglobulin,** is usually associated with serum-albumin, from which it may be separated. It may be detected in the urine as follows: To a large volume of water in a beaker or urine-glass let fall a few drops of albuminous urine. If globulin be present, each drop as it falls will be followed by a milky train, which, when enough is added, forms an opalescent cloud in the water. The addition of acetic acid dissolves this cloud. This test depends upon the fact that globulin is soluble in a weak solution of sodium chloride, such as urine is, but on greatly diluting this solution the globulin becomes insoluble. It is, therefore, precipitated by diluting the urine until the specific gravity is 1002 to 1003.

It may be precipitated by rendering the urine slightly alkaline with NH_4OH, filtering to separate the phosphates, and adding to the fil-

trate an equal volume of a saturated solution of ammonium sulphate. If a precipitate forms, it is globulin. It occurs with serum-albumin, and rarely without it. It is most abundant in lardaceous kidney, in some cases of acute nephritis, and in the temporary albuminuria of disordered digestion.

Albumoses, or Propeptones. — To test for albumose it is best to first remove the albumin. This is best done by acidifying the urine with a few drops of acetic acid and adding an equal volume of a saturated solution of common salt, boiling and filtering while hot. Albumin and globulin are thus removed. (1) The filtrate is allowed to cool, and any turbidity which separates on cooling, and which disappears on heating to reappear again on cooling, is albumose or propeptone. This precipitate will appear only when albumose is present in considerable quantity. (2) This filtrate may be further examined as follows: Half fill two tubes with the filtrate. To both of them add one c.c. of a saturated aqueous solution of salicyl-sulphonic acid.

A turbidity will indicate albumin, globulin, nucleo-albumin or albumose. Heat one of the tubes to or near to boiling and compare the tubes. If the turbidity be due to albumose this will have been dissolved in the hot tube, and there will be a diminished turbidity in that tube.

The albumose precipitate is completely dissolved on adding an excess of ammonium hydroxide, while the albumin and globulin precipitate is insoluble.

When the original urine contains no albumin or globulin, this test may be applied directly to it.

It is best, however, to remove any nucleo-albumin that may be present by the addition of a few drops of lead acetate, taking care not to add an excess, filtering out the heavy white precipitate, and applying the test to the clear filtrate.

(3) The albumin and globulin, if present, may be removed by the addition of some sodium acetate solution, then enough ferric chloride to give a deep red color, nearly neutralizing with dilute NaOH solution, heating to boiling and filtering clear. A small portion of the filtrate is treated with a few drops of acetic acid and then with potassium ferrocyanide. If a blue color appears, showing the presence of iron in the filtrate, the whole filtrate is made slightly alkaline, warmed again and filtered.

To 100 c.c. of urine, thus freed from albumin or globulin, add 5 c.c. of strong HCl, and then phosphotungstic acid as long as a precipitate forms. Warm the solution on a water-bath until the precipitate settles to the bottom of the vessel. Pour off the clear

supernatant liquid and add distilled water, shake up and again allow the precipitate to settle. Repeat the process once more. The precipitate is dissolved in NaOH solution, and the solution is heated until the blue color disappears. The solution is now tested by the biuret reaction. A rose-red color indicates albumose.

Clinical Significance. — Albumose in the urine is a valuable sign of concealed pus somewhere in the body. Whenever there exists pent-up pus, not walled off by adhesions, the disintegration of the pus corpuscles gives rise to albumose in the urine. Its diagnostic importance is therefore very great.

It is found during uterine involution after labor, whether there be suppuration or not.

It is found in cerebro-spinal meningitis but not in tubercular meningitis, in pneumonia during resolution, in purulent pleurisy, suppurating appendicitis, but ceases when the abscess is walled off by adhesions, during typhoid fever when intestinal ulcers have formed, during the formation of cavities in phthisis, in abscess of the liver, peritonitis, carbuncles, pelvic abscess, etc.

Peptonuria. — True *peptone* does not occur in normal urine, and rarely in pathological urines. What is sometimes spoken of as peptonuria is properly albumosuria.

Nucleo-albumin and Mucoid Substance. — This is a compound proteid found in traces in normal urine and is secreted by the epithelium of the urinary tract. It is often confounded with *mucin* which is a gluco-proteid, and which on boiling with dilute acids yields a reducing sugar in greater abundance than mucoid substance. Both bodies are precipitated with acetic acid, but mucoid is soluble in excess while mucin is insoluble. According to Mörner normal urine contains no mucin, but the flocculent sediment seen in most urines on standing a short time, is composed mostly of **mucoid substance.** In some pathological conditions true mucin may be found in the urine.

Nucleo-albumin is characterized by giving a precipitate with acetic acid (soluble in excess) and with $MgSO_4$ added to saturation. The precipitate is soluble in weak NH_4OH.

1. To 10 c.c. of the clear filtered urine, free from albumin and globulin, add strong acetic acid drop by drop. An opalescence or turbidity will indicate nucleo-albumin or mucoid substance. The presence of the neutral salts hinder this precipitation. It is best, therefore, to dilute the urine with three volumes of water before making the test.

2. **Ott's Test.** — To 5 c.c. of the clear urine, add an equal volume of saturated solution of NaCl, and then 1 or 2 c.c. of Almén's solution. An immediate precipitate indicates nucleo-albumin. *Almén's solution:* 5 grms. of tannic acid, 10 c.c. of a 25 per cent. acetic acid and 240 c.c. of 45 per cent. alcohol.

3. **Heller's Test.** — Nucleo-albumin gives a white zone or diffused cloud, similar to albumin, but from 0.5 to 1 centimeter above the acid. This cloud is often more distinct in the diluted (1 to 3) urine.

4. To 5 c.c of the urine add a distinct excess of acetic acid and then 1 c.c. of chloroform, or 2 c.c. of alcohol, and agitate. Nucleo-albumin is precipitated.

5. To distinguish between nucleo-albumin and mucin, precipitate as in (4), filter, dissolve the residue on the filter in a few c.c. of dilute NaOH, poured through the filter. Acidify this filtrate with HCl, boil for two or three minutes, make alkaline again and heat with a few drops of Fehling's solution. Mucin reduces the copper, while nucleo-albumin does not. Neutral lead acetate precipitates both substances from urine.

Histon and **nucleo-histon** have been found in urine, but their significance is doubtful.

Clinical Significance of Nucleo-albumin. — It is usually found in urine contaminated with vaginal secretion. Also in catarrhal conditions of the urinary tract. It has been found in urine of leukemia, diphtheria, scarlatinal nephritis, cystitis, and after the use of irritating drugs, as mercuric chloride, naphthol, pyrogallol, turpentine, etc. Most concentrated febrile urines and urines containing bile contain it.

The separation of the various proteid substances found in urine.

Filter the urine clear. To 25 c.c. of the clear urine add a few drops of strong acetic acid drop by drop.

A precipitate indicates *nucleo-albumin*, *mucin*, or *mucoid substance*. Filter.

Saturate a portion of this filtrate with $MgSO_4$ or NaCl. A precipitate indicates *globulin* or *fibrin*. Filter.

Boil the last filtrate. A precipitate indicates *albumin*. Filter.

Saturate the filtrate with $(NH_4)_2SO_4$. A precipitate indicates *albumose* or *propeptone*. Boil, saturate the hot solution with $(NH_4)_2SO_4$ and filter while still hot. Allow the filtrate to cool, when some of the $(NH_4)_2SO_4$ will crystallize out. Pour off a portion of the clear liquid and apply the biuret test for peptones. Rose-red color indicates *peptone*. Or, acidify with acetic acid and add phosphotungstic acid. A white precipitate soluble on warming and reprecipitating on cooling indicates peptone.

Fibrin may be found in the urine when there are hemorrhages from the genito-urinary passages, and in intense or acute inflammations of the kidneys. It also occurs in the urine of most cases of chyluria. It is readily recognized by its spontaneous coagulation, forming a thick, gelatinous, glairy mass, separating at the bottom of the containing vessel. The coagulum may be filtered out and its solubility determined. If insoluble in dilute alkalies and 10 per cent. NaCl solution, it is probably fibrin.

The table on page 77 from Allen's Commercial Organic Analysis, slightly modified, shows the behavior of the urinary proteids with the usually employed reagents:

Hematuria and Hemoglobinuria. — In this condition the urine usually has a red or brown color, and deposits a red sediment. Hematuria can not be regarded, usually, as a disease, but as a symptom of some other disease. Nevertheless, it is very important that we should be able to determine the source of the blood—whether it comes from the kidneys tubules, the ureters, pelvis of the kidney, bladder, urethra, or possibly from the rectum or vagina.

We may divide the hematurias, for convenience, into the following classes: (1) **Ordinary hemorrhage from rupture of blood-vessels;** (2) **parenchymatous hemorrhage,** or slow escape of blood through

Behavior of Urinary Proteids with Reagents.

	Serum albumin.	Serum Globulin.	Heteroalbumose.	Deuteroalbumose.	Peptone (?,.	Acid Albumin.	Alkali Albumin	Mucin.
Dilution with water..	No change.	Slight opacity.	No change.	No change.	No change.	No change.	No change.	No change.
Saturation with $MgSO_4$.	No change.	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	.....
Saturation with $(NH_4)_2SO_4$.	Precip tate.	Precipitate.	Precipitate.	Precipitated but not wholly.	No change.	Precipitate.	Precipitate.	.....
Boiling and acidulation with $HC_2H_3O_2$.	Precipitate.	Precipitate.	No change.	No change.	No change.	No change.	Precipitate.	No change.
Cold concentrated HNO_3.	Precipitate.	Precipitate.	Precipitate soluble in excess, or on heating, reappears on cooling.	Precipi t a t e d only on adding salt. Dissolved on heating, reappearing on cooling.	No change.	Precipitate.	Precipitate.	Precipitate.
Metaphosphoric acid.	Precipitate.	Precipitate.	.....	.....	.....	No change.	Precipitate.	.....
Picric acid.....	Precipitate.	Precipitate.	Precipitate soluble on heating.	Precipi ate soluble on heating.	Precipitate soluble on heating.	Precipitate.	Precipitate	No precipitate until acetic acid is added.
Potassium ferrocyanide.	Precipitate,	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	.....
Potassium mercuric iodide	Precipitate.	Precipitate.	Precipitate.	Precipitae soluble on heating	Precipitate soluble on heating.	Precipitate.	Precipitate.	Precipitate.
Fehling's solution (biuret test).	Brown-red or violet color.	Brown-red or violet color.	Rose pink color.	Rose-pink color.	Rose-pink color.	Brown-red or violet color.	Brown-red or violet color.	Violet.
$CuSO_4$.....	Precipitate.	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	.....

the epithelium of the tubules of the kidney; (3) **hemoglobinuria,** or **hematinuria.**

1. In this variety the urine is dark-red in color, similar to venous blood. Reaction neutral or alkaline; rapidly deposits a sediment, leaving the clear urine above; we often find clots or fibrinous masses; specific gravity variable, but usually high; albumin always present.

2. Reddish-brown, often coffee-colored; retains its uniform color for a long time, but finally deposits a red sediment; generally acid; specific gravity variable. It contains altered hemoglobin or methemoglobin. The sediment is characteristic, when examined microscopically. The blood-corpuscles are not distributed in *rouleaux*, or rolls. Some of them are changed in size; some spherical and colored brown, or sometimes quite colorless. We often observe corpuscles only one half or one quarter of the usual size, and from that down to mere granules, called **microcytes.** They are considered characteristic of parenchymatous hemorrhage from the urinary passages.

3. Hemoglobinuria (Vogel's hematinuria) is also called dissolved blood. The urine is usually quite dark in color, smoky or even black. The coloring matter is in solution, the urine retains its color, and does not deposit a sediment of corpuscles. Reaction acid; specific gravity lowered. Usually met with in typhoid or typhus fever, malarial infection, and in smelters suffering from inhalation of arseniuretted hydrogen.

Detection of Blood. — The presence of **blood** may be detected most readily and certainly by the microscope, when the red blood-corpuscles may readily be seen, or by the spectroscope (p. 30).

Guaiacum Test for Blood. — Mix a small portion of the urine in a test-tube with an equal volume of a mixture of freshly prepared tincture guaiacum and spirits of turpentine. The turpentine should previously have been exposed to the air for some time. If blood-coloring matter be present, the mixture assumes an indigo-blue color, whose rapidity of formation and depth of color depend upon the amount of blood-coloring matter present. From the depth of the color of the urine, and the rapidity of the appearance of the blue color, one can judge of the relative amount of blood present. The spirits of turpentine used in this test may be replaced by a solution of peroxide of hydrogen, or a mixture of ether and H_2O_2 (ozonic ether). Pus frequently, if not always, gives the same color. Saliva and salts of iodine also give a blue color with this test, but the color due to these substances appears only after a considerable lapse of time, and is seldom likely to mislead.

Precipitation Test. — If we add an alkali to urine containing blood,

the earthy phosphates are precipitated, carrying down with them the blood-coloring matter and forming a blood-red deposit. By the application of heat the sediment deposits more rapidly, and the solution may assume a green color. If the urine is already alkaline, and the phosphates have separated out, we can produce a precipitate for the purpose of carrying down the blood-coloring matter by the addition of a few drops of a solution of $MgSO_4$ and warming.

Hemin crystals may be prepared from the above precipitates, by spreading a small portion of them upon a glass slide, and treating this with a crystal of common salt and a drop or two of glacial acetic acid, covering with a coverglass, warming it gently, and examining, after a few hours, with the microscope. The crystals appear as small, oblique plates of a dark-red or brown color. They are easily recognizable by a good ¼-inch lens.

Having determined that blood is present in the urine, it is a difficult matter to decide whether the albuminuria is due entirely to the albumin introduced with the blood, or whether it is a true albuminuria of renal or inflammatory origin. This will often depend upon other symptoms than those to be found in the urine. Dissolved blood coloring matter is sometimes met with in the urine, when it is called **hemoglobinuria.** In hemoglobinuria, blood-corpuscles are not to be found with the microscope, while in **hematuria** the corpuscles are found. It occasionally happens that the urine rapidly becomes alkaline after being secreted, and the red blood-corpuscles are disintegrated and dissolved by the alkaline urine. The urine containing the dissolved corpuscles is then always alkaline, while the urine of true hemoglobinuria is usually acid. We may conveniently distinguish, then, two conditions: In one, the blood-coloring matter is in solution, and in the other it is in suspension in the blood-corpuscles. In the former case the coloring matter will not separate on standing, while in the latter there will usually separate, within a few hours, a more or less abundant red sediment. If the hemorrhage be a profuse one, especially if from the bladder or ureters, the blood will almost all of it settle to the bottom of the containing vessel, and leave a clear yellow, almost normal-looking urine above; while if the hemorrhage be a gradual oozing, as in acute inflammation of the kidneys, the coloring matter will remain in suspension and the liquid retain its color for many days. It is not unusual to have hemoglobinuria and hematuria together, especially in acute diffuse nephritis, or in malarial hematuria.

Pus. — Urine containing **pus** will always be turbid to the naked eye, and rapidly deposit a white or greenish-white sediment. The clear solution will be found to contain albumin, globulin, and usually albumose. The application of heat does not dissolve this sediment, as it does the sediment of urates. Neither is it dissolved by dilute acids, as is the somewhat similar-looking precipitate of the earthy phosphates.

Donné's Test. — Add to the white sediment twice its volume of NaOH solution and mix well by shaking. If the sediment be pus, it will become translucent instead of opaque, and gelatinous, resembling white of egg. A whitish sediment, therefore, which is insoluble with heat or dilute acids, and which dissolves in strong alkaline solutions,

giving a gelatinous, ropy liquid, is probably pus. When pus is treated with a solution of hydrogen peroxide it undergoes rapid effervescence. This is a valuable test for pus in the urine or in other fluids. The microscope is a more certain test for pus. The origin of the pus must be determined by a careful search for and identification of the epithelial cells which accompany it. Having detected pus in the urine, it is sometimes very difficult to determine whether the albuminuria accompanying it is accidental — *i. e.*, whether the albumin is derived from the pus, or whether there is a true albuminuria due to some disease of the kidney. The symptoms of the patient will assist in many cases, though not in all.

Significance of Albuminuria. — Albumin may appear in the urine in a variety of conditions.

Some years ago albuminuria was looked upon as evidence of a serious, diseased condition. In recent years it has been very much discussed whether it may not occur in, and be compatible with, good health. It has been claimed by some that albumin may occur temporarily in the urine of certain persons who are in a state of normal physiological health. This form of albuminuria has been called by them **physiological** or **functional albuminuria.** In some persons the albumin may appear at intervals, and then disappear to reappear again. This form of albuminuria has been called **cyclic albuminuria,** or **intermittent albuminuria.** Most authors regard all forms of albuminuria as abnormal, but they admit that it may appear in cases where no other symptoms of disease can be made out. It is admitted by all that albumin does not appear in the urine as a regular every-day occurrence and without cause. It is certain that very slight causes are sometimes sufficient to determine its appearance; and yet, a normal kidney, without any disturbing element, does not allow albumin or globulin to pass into the urine. The disturbing element is often so slight that many authors are not willing to admit that it constitutes a pathological condition. Such, for example, are: severe muscular exertion, a cold bath, mental exertion, severe emotons, menstruation, during digestion, etc.

Gireat care should be exercised in making a diagnosis of physiological albuminuria. For such a diagnosis the amount of albumin must be small, not exceeding one tenth of the volume of the urine tested; no tube-casts should be present, the amount of urea should be fully up to normal, there should be no retinal changes, no hypertrophy of the left ventricle, no dropsy, and no abnormal pulse-tension.

The albumin in such cases is usually absent from the urine passed on rising in the morning, although this is not absolutely necessary. When this does occur, it is a strong point in favor of the diagnosis of functional albuminuria. Such cases should be watched for some time before we decide that we are dealing with a case of "functional albuminuria."

The association of albuminuria with an increased elimination of uric acid and calcium oxalate has been noticed by some authors. While such albuminurias may be only temporary in character, the conditions under which they occur can not be regarded as physiological, although they are not pathological in the sense that there is any evident organic change in the kidney structure.

We may conveniently classify the different forms of albuminuria as follows:

1. *Functional albuminuria*, which has already been described.

2. *Febrile Albuminuria.* — Under this head we would place the appearance of albumin in the course of many of the specific fevers, not dependent upon a true

nephritis. The albumin usually appears at or near the acme of the disease, and it disappears during convalescence. In typhoid fever, for example, it is to be expected that albumin will make its appearance in the urine during the height of the fever, and Robin, who has studied this subject, says that it appears in all cases at some time. My own experience does not confirm this statement. I have seen cases in which I have not been able to find it at any time during the course of the fever. As to the cause of the albuminuria, opinions differ. Perhaps it would be better to say that it may be due to several causes, and each of these have been magnified by some one author. The causes that may be mentioned are: Changes in the blood-tension; the irritation of the bacterial poisons; the irritation of the concentrated, highly acid urine, containing an excess of urea, uric acid, and extractive matters; or changes in the composition of the blood itself.

The following notes from Robin's work on "The Urine of Typhoid Fever" will give an idea of the occurrence of albuminuria in other fevers:

Pneumonia: Albumin is usually present as in typhoid. At times abundant, especially in severe cases.

Acute miliary tuberculosis: Albumin not so constant as in typhoid. When present, it is not so abundant as in the severe or fatal cases of typhoid.

Epidemic influenza; grippe: Albumin present in traces in about twenty per cent. of the cases.

Gastric fever: Albumin seldom present.

Herpetic fever; urticaria: Never present in more than traces.

Rubeola: Present only in the severer cases.

Scarlatina: Albumin present in a considerable number of the cases, but usually by virtue of a veritable nephritis.

Enteritis in adults of the adynamic type: Traces usually present.

Cerebro-spinal fever: Albumin present in fairly large amount.

Vegetative endocarditis: Albumin usually present in variable amount.

Acute rheumatism: Albumin present in about forty per cent. of the cases.

Intermittent fever: Albumin present in some cases, but is not constant.

In short, it may be said that albumin may be present in all fevers.

3. *Albuminuria Due to Circulatory Disturbances.* — To this class belong those resulting from cardiac insufficiency, due to valvular disease or dilatation, leading to renal hyperemia. The pressure of abdominal tumors or of a gravid uterus, violent exercise, and other causes of disturbed renal circulation may cause albuminuria.

4. *Toxic Albuminuria.* — This has already been referred to as a possible explanation of the albuminurias of the specific fevers. Some authors claim that the albuminuria of pregnancy is due in part, at least, to changes in the blood during this condition. Some of the functional albuminurias are due to changes in the composition of the blood, and Semmola and others believe that in Bright's disease the first change is a blood change. Clinically, we observe albuminuria of toxic or hemic origin in scurvy, purpura, leukemia, pernicious anemia, and in poisoning with cantharides, mustard, oil, turpentine, carbolic acid, salicylic acid, petroleum, lead, mercury, copper, iodine, phosphorus, arsenic, antimony, alcohol, the poison of syphilis, uric acid, diabetes mellitus, and after inhalations of ether and chloroform.

5. A *neurotic albuminuria* has been described by some. Found after epileptic seizures, in delirium tremens, neurasthenia, migraine, and Basedow's disease (exophthalmic goiter). This may be a useful test for malingerers. (Senator.)

6. A *digestive albuminuria* must be recognized. This is occasionally seen after the free indulgence in eggs or beef, and it has been seen after drinking freely of root-beer and ginger-ale. English and Frank found albuminuria in two thirds of the cases of obstruction of the bowel, the quantity being in proportion to the severity of the disease. This disappeared with the relief of the obstruction.

7. *Albuminuria from Organic Disease of the Kidneys.* — In acute nephritis albuminuria is constant, but the amount is subject to considerable variation. The quantity is usually proportional to the intensity of the disease, from a daily amount of from 5 to 15 or 20 grms.

8. *Accidental Albuminuria.* — By this term we will designate albumin added to the urine with pus, blood, leucorrhœal discharge, blennorrhœa, spermatic fluid, lymph, or chyle. Whenever albumin is found in the urine, it becomes an important matter to determine its source and meaning. This is not always an easy matter. We must also remember that we may have a mixed origin of the albumin; a part of it may come from the kidneys, and a part of it may be accidental. In most cases a careful microscopical examination will reveal its source. Pus, blood, vaginal discharge, spermatic fluid, urethral discharge, and chyle will be made evident at once. In cases of doubt it will be necessary to obtain the urine with a catheter, or even by catheterization of the ureters. We can frequently eliminate leucorrheal and urethral discharges by instructing the patient to pass a part of the urine before collecting the sample, so as to flush the passage and wash out the mucus and pus.

Serum-globulin frequently occurs together with albumin, in cases where the latter appears, although in smaller quantities. In amyloid degeneration, according to Senator, the proportion of globulin to albumin is much greater than in any other disease. In this disease, he says, the two forms occur in nearly equal quantities, and he thinks such a ratio is fairly constant and of diagnostic importance.

DIAGNOSIS OF RENAL DISEASES ACCOMPANIED BY ALBUMINURIA.

Our knowledge of diseases of the kidney dates back to 1827, when Richard Bright, of Guy's Hospital, London, described the disease. These diseases are usually classed under the indefinite title of "Bright's disease." This title is not sufficient. There are at least six varieties, and some say twelve. We may, for our purposes, classify them as follows: Acute and chronic congestion, acute and chronic parenchymatous nephritis, acute and chronic diffuse nephritis, chronic interstitial nephritis and amyloid kidney.

Acute Congestion, or Hyperemia. *Definition.* — An abnormal influx of arterial blood, temporary in character.

This may be due to irritation or paralysis of vasomotor nerves, the first stage of acute nephritis; the poisons of various infectious diseases, as scarlatina, small-pox, measles, etc.; exposure to cold and malarial attacks (least common); irritating diuretics, as potassium salts, cantharides, etc.

Urinary Symptoms. — Those of the exciting disease. When due to cold or irritating diuretics, there may be a chill or strangury, great pain in loins or hypogastric region, followed by almost total suppression. Urine has a high specific gravity and high color, and may or may not contain albumin or blood. As recovery begins, the first urine passed is loaded with urates. The symptoms may last a few hours or several days. The urine may contain blood or hyaline and blood-casts. Prognosis generally favorable. Occasionally fatal, from continued suppression.

Passive or Chronic Congestion (*Cyanotic Induration*).

Causes. — Any mechanical cause which prevents escape of blood from the kidneys by renal veins, as emphysema, pericarditis, hydropneumothorax, mitral insufficiency. The most common causes are, aneurysm of the arch of the aorta, dilatation of the right heart, or mitral insufficiency.

Diagnosis. — The diagnosis will rest upon the evidences of obstructed venous circulation, and upon the urinary changes. In chronic congestion the urine is much decreased in quantity, with a high specific gravity (1025 to 1030), a dark color, and often loaded with urates. Albumin and blood-corpuscles are quite common. Casts are infrequent, but occasionally a few hyaline, finely granular, or blood-casts may be present. Albumin usually present in small amount. The value of $(\Delta V) \div P$ is below normal and of $\Delta \div \delta$ is high. (See p. 106.) If the obstruction increases, the urine becomes less and less, until it amounts almost to suppression. Uremic symptoms will then appear, with headache, convulsions, or coma.

If congestion continues, it ultimately leads to organic changes and permanent nephritis.

Acute Parenchymatous Nephritis.

Definition. — A disease affecting the epithelium of the tubules, by which the cells become swollen and granular, or detached. Kidney enlarged and light-colored. In mild cases the convoluted, in severe cases the convoluted, the straight tubes, and glomeruli are affected. Albumin is present in the urine in all cases.

Urinary Symptoms. — *Idiopathic Cases, or those due to Exposure.* This is essentially the nephritis of acute fevers. Urine diminished or suppressed. Specific gravity nearly normal. Albumin usually large, often blood. Casts sometimes few, sometimes abundant. Hyaline-, blood-, granular-, and epithelial casts.

Secondary Cases. — Urine usually diminished in quantity, albumin abundant; sometimes contains blood. Hyaline and granular casts. Dropsy in post-scarlatinal variety, usually not in others. Cerebral symptoms occur in the severe cases. *Duration* in idiopathic cases is short, where dropsies occur. Secondary cases apt to last for some weeks after primary disease disappears.

Acute Diffuse Nephritis.

Synonyms. — *Acute desquamative, scarlatinal nephritis, acute tubal, croupous, glomerulo-nephritis, acute interstitial nephritis,* or *acute Bright's disease.*

Definition. — An acute inflammation of toxemic origin, and involving practically all the structures of the kidney.

Urinary Findings. — The urinary examination alone will not, as a rule, distinguish between this and the purely parenchymatous variety of the disease. The quantity of urine is diminished, or may be entirely suppressed. Color dark, smoky, chocolate or blood-red. Hemoglobinuria often present. Red blood cells well distributed. Albumin constant and abundant. Globulin usually present; proteoses and nucleo-albumin often present.

Total solids, urea, sodium chloride and phosphates are diminished in very acute stage; urea and solids decreased in all stages. Purin bodies increased.

The sp. gr. increased at first, but later diminished as quantity of water increases.

Sediment abundant, rendering the urine turbid and of dark-red color; contains casts of great variety and size; blood cells, red and white, leucocytes, renal epithelial cells, free and imbedded in casts. There are hyaline, epithelial, blood and granular casts, all more or less stained with hemoglobin, and of all possible sizes.

Chronic Parenchymatous Nephritis. — *Chronic Bright's, Large White Kidney, Chronic Diffuse Nephritis, Croupous Nephritis, Catarrhal Nephritis, Small White Kidney, Chronic Parenchymatous with Glomerulo-Nephritis.*

Definition. — A chronic, diffuse, hyperplastic process in one or both kidneys involving the epithelium, glomeruli and interstitial tissue. There are two stages: (1) Stage of enlargement and (2) stage of contraction.

Urinary findings. — During the stage of enlargement the quantity of urine is less than normal. It increases as the disease improves and becomes more than normal in the contracting stage, until late in its course, when it again diminishes, near the fatal termination.

The urine is often turbid, and albumin is constantly present and in large amount. It may form a heat-coagulum occupying one half to three fourths of the volume of urine tested, or from 0.5 to 2 per cent.

The sp. gr. is usually if not always lower than normal. In the contracting stage the gravity is low and the color light, and the urine is more or less turbid.

The total solids, including urea, are generally diminished. The electric conductivity is lower than normal and the freezing point is higher than normal. The factor $(\Delta \times V) \div P$ (see page 106) is less than 3,000, and the value of $\Delta \div \delta$ is above 1.7 in proportion as the function of the cells of the tubules diminishes, and may rise to 2.5 in approaching uremic crises or death.

In the later stages the sediment often becomes abundant and shows

a large amount of granular detritus. The indican is usually increased.

The casts are at first hyaline and granular, later densely granular and of variable sizes, often very large. Fatty casts are common, and waxy casts may be found. Renal cells are usually present, but many of them will be partially broken up and disintegrated. A careful examination of these fragments of cells, and an estimate of their number, will give valuable information as to the amount of organic destruction going on in the kidney, and is a valuable indication in prognosis. Leucocytes are abundant, and erythrocytes may be found, but not as a rule.

Chronic Interstitial Nephritis. *Synonyms. — Cirrhotic Kidney, Contracted Kidney, Gouty Kidney, Red Granular Kidney, Chronic Productive Interstitial Nephritis, etc.* — This disease is a chronic inflammation of the kidneys, with hyperplasia of its connective tissue, with subsequent contraction. It is usually, if not always, bilateral.

Urinary Findings. — The *urine* is increased in quantity, and the specific gravity is usually low — 1005 to 1010. There is usually but a trace of albumin, and occasionally it is absent. A few hyaline casts will usually be found, but they often require careful search.

The symptoms upon which a positive diagnosis may be made are, a large quantity of urine of persistent low specific gravity, with diminished urea, chlorides, and solids, with a small quantity of albumin and a few hyaline casts, persisting for months or years, and accompanied by increased arterial tension and hypertrophy of the left ventricle.

The urine is usually clear and transparent and seldom deposits a visible sediment.

The quantity may reach 90 ounces (2,700 c.c.), and the sp. gr. is usually between 1005 and 1015. Toward the termination of cases of this disease, the quantity diminishes, the amount of sediment and casts become more abundant, and the casts become granular. The specific gravity may increase during this stage or it may remain low.

Throughout the disease the factor $\Delta \div \delta$ (p. 106) is high, and it increases towards the termination of the disease. This ratio varies, however, from time to time, as the renal permeability changes, without any observable change in the microscopic findings in the urine.

Renal Amyloid Degeneration. *Synonyms. — Waxy Kidney, Amyloid Kidney, Lardaceous Kidney.*

Definition. — This is a peculiar degeneration of the various structures of the kidney, the result of a toxemia, and accompanying chronic suppurative conditions, tuberculosis, syphilis, or other cachexia.

Urinary Findings. — Quantity regularly and considerably increased, sp. gr. 1005 to 1012, little sediment, albumin constant and rather

abundant, a few hyaline or waxy casts. The total solids are usually about normal, until late in the disease. It is very apt to be associated with parenchymatous nephritis. The differential diagnosis between amyloid and interstitial degeneration by the urine alone is often impossible.

In uncomplicated amyloid degeneration the liver and spleen are enlarged, and there is an absence of the high arterial tension and cardiac hypertrophy found in interstitial nephritis.

Pyelitis. — This is a disease characterized by suppuration in the pelvis or tubules of the kidney, and is usually unilateral. Both kidneys may be involved. The mucous membrane of the pelvis alone may be affected, or it may involve other structures, giving rise to *pyelonephritis*. When cystitis is also present, it is called *pyelocystitis*. When the ureter becomes obstructed so that the pus cannot escape, the kidney becomes distended, and it is called *pyonephrosis*.

The Urine (see page 131).—The urine is cloudy in the first three of these affections, and rapidly forms a white sediment of pus-cells. The reaction in pyelitis and pyelonephritis is acid; the specific gravity is normal in the first, and normal or less than normal in the second. In pyelocystitis the reaction is alkaline in most cases, and the specific gravity is normal or lower. Albumin and pus are present in all three affections, as is also globulin. In pyonephrosis the pus and albumin may occur in the urine at times, but may at other times be absent. If a calculus be the cause of the pyelitis, more or less blood may be found in the urine. In the earlier stages the peculiar caudate or spindle-shaped epithelial cells from the pelvis will be found, but later in the disease these may be absent.

Acute Cystitis.

Causes. — Traumatism, gonorrhea, infection from a catheter, calculi, retention of urine, pyelitis, very acid urine, uterine displacements.

The urine is lessened in quantity, cloudy, neutral or alkaline in reaction, and sometimes becomes putrid in the bladder. It contains numerous epithelial cells from the bladder, and in very acute cases more or less blood, pus and bacteria.

Chronic Cystitis. — This may follow the acute form, or it may come on gradually. It is usually due to some obstacle to free voiding of the urine, as stricture of the urethra, prostatic enlargement, vesical calculus, etc., or to infection from a catheter. It may result from a prolapsed or anteverted uterus in females, and tumors, either benign or malignant.

Urinary Symptoms. — *The urine* is usually alkaline, viscid, turbid, and ammoniacal or putrid; the quantity and the specific gravity are

usually below normal. The urine contains an excess of nucleoalbumin, pus-corpuscles, an abundance of epithelial cells from the bladder and crystals of ammonium magnesium phosphate. When the cystitis is due to a calculus, blood corpuscles will at times be found, or there may be marked hematuria. When due to enlarged prostate, there will always be a considerable residual urine in the bladder, even after the patient urinates.

Chronic cystitis is apt, sooner or later, to extend upward through the ureters, and produce catarrhal nephritis or pyelonephritis, described above.

Diagnosis. — Cystitis is likely to require differentiation from *prostatitis*. This affection may be either acute or chronic, and the symptoms resemble those of cystitis. There is frequent and painful micturition, the pain being in the perineum and of a throbbing character. There is less control of the desire to urinate than in cystitis. The pain is worse at the close of urination. The urine in prostatitis contains excess of mucus, with mucous casts of the follicles of the prostate.

CARBOHYDRATES.

Normal urine, contains traces of carbohydrates of which at least three are known: Animal gum, dextrose and isomaltose. Besides these, pentoses are frequently met with after taking certain foods or drinks. To these may be added certain substances which contain a carbohydrate group which can be split off by reagents. These are glycuronic acid, the glucoside, chondroitin-sulphuric acid, and mucoid substance of mucus. In pathological conditions, dextrose, levulose, lactose, sucrose or cane-sugar, erythrodextrin and even glycogen have been found.

By means of the alpha-naphthol (furfural) test, it has been found that normal urine contains from 0.075 per cent. to 0.35 per cent. of reducing substances, or in 24 hours from 2 to 2.82 grms., calculated as dextrose.

Many of these substances are present in such small quantity as to show no reaction with the ordinary reagents, while others readily reduce some of these solutions.

The most important carbohydrate, from a clinical standpoint, is *dextrose* or as it is often called *glucose*.

Dextrose. — As above stated this sugar occurs in traces in most normal urines, but not in sufficient quantity to be detected by any of the following tests, except Molisch's, No. 11. Its appearance, then, in sufficient quantities to be detected by any of the others must be regarded as abnormal.

When glucose occurs in the urine in an appreciable amount, it is known as **glycosuria.** When its occurrence persists for a considerable time and in considerable amount, and is attended with an increased amount of a light-colored urine, generally of a high specific gravity, it is pathological, and the disease is known as **diabetes mellitus.** The specific gravity is some guide to the detection of diabetes mellitus, but the specific gravity alone is not conclusive. A persistent high specific gravity, with a large quantity of light-colored urine, is strong presumptive evidence of diabetes mellitus. The finding of sugar in such a case is confirmatory.

Tests for Glucose. Trommer's Test. — To 4 or 5 c.c. of urine, in a test-tube, add one half its volume of sodium hydroxide solution, and 4 or 5 drops of a solution of $CuSO_4$ (1 to 10). If sugar be present, a clear, deep-blue solution is obtained. The solution is now to be heated almost to boiling, but it is better not to boil. If sugar be present, at first a greenish and then a yellowish turbidity forms, which rapidly changes to a reddish yellow color, and precipitates red cuprous oxide.

A flocculent precipitate of the earthy phosphates always forms on adding the alkali, and must not be mistaken for suboxide of copper. Urine containing uric acid, the xanthin bases, creatinin, indoxyl-sulphates, lactose, glycuronic and glycosuric acids, mucus, and other substances found in some urines will decolorize the blue solution, but there will be no red precipitate. In fever urines, this decolorization without precipitation interferes greatly with the employment of this test. *It is, therefore, not to be relied upon in doubtful cases.*

To eliminate this source of trouble with the copper test, it is best to precipitate the uric acid, xanthin, hypoxanthin, and the most of the creatinin and phosphates as follows.

From 7 to 8 c.c. of the urine are heated to boiling in a test-tube, and, without filtering from any precipitate that may form, adding 1 c.c. of the copper sulphate solution, then, when partially cooled, 1 to 2 c.c. of a saturated solution of sodium acetate, having a slight acid reaction, and filter. To the filtrate, which should have a greenish-blue color, add the alkali, or, better, the alkaline tartrate solution used to make Fehling's solution, and boil for fifteen to twenty seconds. Thus modified, the copper test is much more reliable. Most of the interfering substances may be separated by adding to the hot urine one fifth its volume of a 10 per cent. solution of lead acetate, filtering off the precipitate, and testing the filtrate.

2. **Other Forms of the Copper Test. — Haines' solution** is made by dissolving copper sulphate in a mixture of equal parts of glycerol and water. To 2 c.c. of this solution add 4 c.c. of NaOH solution,

and heat to boiling. Now add 1 c.c. to 2 c.c. of the urine, let set for 5 to 10 minutes and examine for a red sediment. *Do not boil after adding the urine.* The decolorizing effect of normal urine is not sufficient to decolorize a large amount of copper solution. **Fehling's solution** is sometimes employed as a qualitative test, but usually only as a quantitative test; Haines' solution has all the advantages of Fehling's, with the additional advantage that it keeps well.

3. **Böttger's Bismuth Test.** — To a few c.c. of the urine, in a test-tube, add an equal volume of sodium hydroxide and then a fragment of bismuth subnitrate; mix well and boil for from three to five minutes. If sugar be present, black metallic bismuth will be deposited as a sediment. If the quantity of sugar be small, only a part of the bismuth will be reduced and the precipitate will appear gray. Albumin must be removed before this test is applied, or it will be decomposed by boiling with the alkali, forming the sulphide of bismuth, which will give a black precipitate.

4. **Nylander's Test.** — A solution is made of bismuth subnitrate, 2 gm.; Rochelle salt, 4 gm.; sodium hydroxide, 8 gm., and distilled water, 100 c.c. The urine is heated to boiling, a few drops of this alkaline solution of bismuth added, and, on continuing the boiling, if sugar be present, the mixture turns black. As in the previous test, albumin must be absent before this test is applied. This reagent is exceedingly delicate, and is claimed to detect 0.025 per cent. of glucose.

5. **Picric Acid Test.** — To about 5 c.c. of urine add one half as much of a cold saturated picric acid solution (as in testing for albumin), and then 2 c.c. of sodium hydroxide and boil. If sugar be present, a dark, mahogany-red color is developed. If no sugar is present, a dark hue is developed before boiling, but not the dark color above described. If albumin be present, a turbidity will form on the addition of the picric acid, but it does not interfere with the test.

6. **Moore's Test.** — Add to the suspected urine one half its volume of sodium hydroxide solution and boil. If sugar be present, a dark-yellow, brown, or chocolate color is produced. The depth of color is proportional to the amount of sugar present.

7. **Indigo-carmine Test. Reagent.** — Mix one part of indigo carmine, or of dried commercial extract of indigo, with 30 parts of pure dry sodium carbonate. To 5 c.c. of the suspected urine add enough of the above powder to give a transparent, blue solution, and heat to boiling *without agitation.* If sugar is present, the solution changes to violet, cherry-red, and finally yellow. On agitation, these colors appear in the reversed order.

Instead of extract of indigo, a solution of *sulphate of indigo* with an excess of sodium carbonate may be employed. None of the ordinary constituents of the urine affect this test, while many substances occurring in the urine affect Fehling's solution. In careful hands it is to be recommended as a sensitive and reliable test for glucose in the urine.

8. A solution of **methylene-blue** (0.333 grm. per liter) has been used as a test for sugar. Five c.c. of this solution are mixed with 2 c.c. of sodium hydroxide, 2 c.c. of urine added, and the solution is boiled for one minute. If sugar be present, the blue color is discharged, but returns on standing.

9. **Safranin** is another coloring matter used as a test for sugar. Mix equal volumes of sodium hydroxide, safranin solution (1 grm. to the liter of water), and the urine, and heat to boiling. If sugar be present, the red color is changed to a yellow. Uric acid, creatin, creatinin, chloral, and chloroform do not give the test. Albumin must be removed. The test seems reliable.

10. **Phenyl-hydrazin Test.** — For the details of this test see **glucose,** on p. 14.

11. **Alpha-naphthol Test. Molisch's Test.** *Furfural Test.* — To 1 c.c. of the urine add 2 c c. of a 10 per cent. solution of *a*-naphthol in pure methyl- or ethyl-alcohol, and after mixing add an excess of H_2SO_4. If sugar be present, a deep-violet color is developed. On dilution with water a blue precipitate occurs, which is soluble in alcohol, ether, and potassium hydroxide, to give a yellow solution. If, instead of naphthol, we use thymol or menthol, a deep-red color is produced, which gives a carmine-red, flocculent precipitate on dilution, which dissolves as above with the formation of a yellow solution. This test is exceedingly delicate, and reacts with all carbohydrates and most glucosides. Urea, indican, creatinin, xanthin, uric acid, hippuric acid, phenol, and pyrocatechin do not give the reaction. As many normal urines respond to this test, Molisch concludes that they contain sugar.

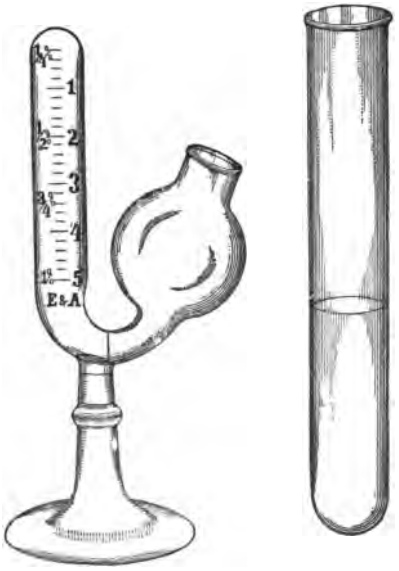


FIG. 10.

The Fermentation Test. — This may be done in a test-tube or with Einhorn's or Lyons' fermentation saccharometer.

Einhorn's instrument is shown in Fig. 10. This may be used as a qualitative or approximate quantitative method. To use this instrument, take about $\frac{1}{16}$ of a cake of commercial compressed yeast (about 1 grm.) and shake up with 10 c.c. of the urine to be tested. Then fill the saccharometer with the urine, expelling all air from the vertical limb by inclining the instrument, and set aside from 20 to 24 hours in a warm room, when the amount of sugar may be read off on the scale. Within an hour the bubbles of CO_2 begin to rise and collect in the graduated part.

Diabetic urines of sp. gr. 1018 to 1022 should be diluted with an equal volume of water and the reading must be multiplied by two. Urines of a sp. gr. of 1022 to 1028 should be diluted with four times their volume, and those of 1028 to 1038 with nine times their volume. In performing this test it is necessary to conduct a blank experiment in another saccharometer with normal urine, using the same amount of yeast. The difference in the readings of the two instruments gives the true reading. Most samples of yeast will give a few bubbles of gas at the end of 24 hours. This precaution is especially necessary when this method is used as a qualitative test. The results are only roughly approximate in quantitative work, and it can not be recommended.

For the *qualitative test* ordinary test-tubes may be used instead of the above apparatus. Select two tubes of the same diameter, half fill one with the urine under examination and the other with water. Add to both about 1 grm. of yeast, shake well and fill with the same liquids in each case. Cork both tubes with a cork having a channel cut in one side, being careful to exclude all air. Invert both tubes in a glass containing a little water, and allow them to stand over night. The dextrose, if present, undergoes fermentation and the CO_2 collects at the upper end of the tube, while a corresponding volume of the urine runs out into the bottle or flask below. The urine should be diluted with water to a specific gravity of about 1015 before adding the yeast. The test depends upon the fact that dextrose splits up under the influence of yeast chiefly as follows: $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. As CO_2 is slightly soluble in urine, it does not all escape from it. For this reason the test is not available when the urine contains less than 0.5 per cent. of dextrose. Yeast will sometimes evolve CO_2 from the fermentation of some of its own constituents. For this reason a check test with pure water and yeast should always be made.

The Quantitative Estimation of Dextrose. — This is generally made with Fehling's solution. This solution is best prepared in two parts, which are kept separate, as the completed solution does not keep well. These solutions are prepared as follows: No. 1, 34.639 grm. of pure, recrystallized copper sulphate are dissolved in distilled water, and made up to exactly 500 c.c. No. 2, 175 grms. of crystallized Rochelle salt and 60 grms. of sodium hydroxide are dissolved in distilled water and made up to exactly 500 c.c. When needed for use, exactly equal volumes of these two solutions are mixed. The solution will be of such strength that 10 c.c. are decolorized by 0.050 grm. of dextrose or diabetic sugar.

Fehling's Method. — Ten c.c. of Fehling's solution are measured out into a beaker or porcelain basin, diluted with about 40 c.c. of water, and brought to the boiling point. The urine is delivered into this blue solution from a burette, until the blue color is just discharged. The amount of urine added is then read off from the burette, and this amount contains 0.050 grm. of sugar. From this it is easy to calculate the quantity contained in 1000 c.c., or a liter. If the urine contains a considerable quantity of sugar, it will be necessary to dilute it with four volumes of water before beginning the tritation, when the results of the titration should be multiplied by five. It is always somewhat difficult to determine the exact disappearance of the blue color, owing to the presence in the solution of the precipitated suboxide of copper. The difficulty may be overcome by the addition of some substance that will prevent the precipitation of the cuprous oxide, as NH_4OH, KCy, or $K_4Fe(CN)_6$.

The Author's Method. — Ten c.c. of Fehling's solution are measured into a suitable flask. To this 10 c.c. of a freshly prepared 10 per cent. solution of potassium ferrocyanide are added, and about 30 c.c. of water. The mixture is heated on a water-bath, and the urine, previously diluted with water, if it contains much sugar, is run in from a burette, drop by drop, until the blue color just disappears. This can readily be seen, as the solution remains clear to the end of the reaction. The

addition of the slightest excess of sugar shows itself by the solution becoming quickly brown. By careful comparative tests the author has found this method to be accurate, reliable, and rapid, provided the solution be not boiled during the reduction. The best temperature for the process was found to be between 85° and 90° C. (176° to 194°F.).

Estimation by the Polariscope. — This is a convenient and rapid method for the determination of glucose, when the quantity exceeds one per cent., and when all the appliances are at hand, which is seldom the case except in well-equipped laboratories. The method, briefly, is as follows: The suspected urine, freed from albumin, is treated with a solution of basic lead acetate, in the proportion of 1 of the latter to 10 of the former, and filtered. The observation-tube of the polariscope is filled with this fluid, when it is placed in position, and the rotation determined.

The readings must be increased by one tenth (allowance for the lead acetate solution).

The specific rotatory power of dextrose is + 52.5°. (See Text Book, p. 52.)

The weight of the sugar in the solution will be given by the formula: $W = \frac{a}{52.5 \times l}$; in which a = observed rotation, l the length of the tube in decimeters, and W the weight of sugar in 1 c.c. of the solution.

Suppose, in a given case, the rotation observed was 4°, after allowing for the lead solution, and the length of the observation tube was two decimeters. We then have

$W = \frac{4}{52.5 \times 2}$, or $\frac{2}{52.5} = 0.038$ grm. in 1 c.c. of urine, or 3.8 per cent.

As *levulose* sometimes occurs with dextrose in cases of diabetes, and as it will rotate the plane of polarized light to the left instead of to the right, and, in fact, as there are a number of substances likely to occur in the urine which rotate the plane of polarized light, this method of determining sugar is not free from error.

Levulose frequently occurs in diabetic urine. It may be detected by the brilliant red color produced when the urine is heated with an equal volume of strong HCl and a few crystals of resorcin. Its significance is not well understood.

Lactose or **milk-sugar,** occurs in the urine of nursing women, or of women soon after weaning. Its recognition requires first its separation from the fluid.

Dextrin has been found in the urine of diabetics, where it seems to take the place of dextrose. Other carbohydrates found rarely in the urine are pentoses, isomaltose, and animal gum. The clinical import of *cane-sugar, animal gum, isomaltose, levulose* is not well known. *Pentoses*, frequently occur in normal and in diabetic urines. The tests given on page 13 will be sufficient to identify them.

Clinical Significance of Glycosuria. — A trace of glucose in the urine has no clinical significance. A larger amount, if temporarily present is known as *glycosuria*, and has no serious meaning. If present in considerable amount, and more or less permanent, it constitutes *diabetes mellitus*. In this disease it is also accompanied by a secretion of a large amount of a light-colored urine of a specific gravity usually above normal, of a sweet taste, and usually containing an excess of urea. Such urines have a viscid appearance, froth easily and often have a sweetish odor. The patient usually suffers from thirst, frequent micturition, loss of weight and strength. No age is exempt, but it is more frequent between the ages of 35 and 60. Less frequent and constant symptoms are cough, tuberculosis, boils and carbuncles, eczema, severe neuritis, most often of the brachial and crural nerves, derangement of the special senses, especially of vision and occasionally of hearing, smell and taste. The fatal termination of diabetes mellitus is most often preceded by coma, the so-called diabetic coma.

Diabetic coma must not be confounded with other forms of coma occasionally met with in diabetes, as, for example, apoplexy and uremia. No doubt many cases which

pass for true diabetic coma are cases of uremia, for, as above stated, sooner or later the kidneys are apt to become structurally deranged.

Sugar is also found in the urine in certain other affections, especially in disturbances of the abdominal circulation. By wounding certain parts of the medulla, temporary glycosuria may be produced in the lower animals. During the first week of lactation and after any obstruction to the flow of milk, as after weaning, there is always a small quantity of sugar in the urine, probably lactose. Sugar has appeared in the urine after internal administration of turpentine, nitrobenzene and nitrotoluene. The liver is continually pouring glucose into the blood to the extent of 1,850 grains per day (4 oz. or 120 gm.), which is burned in the economy. Anything which prevents this oxidation may cause it to appear in the urine. An unusually light-colored or greenish-yellow colored urine, passed in large quantity, and of high specific gravity, should always excite suspicion and lead to a test for sugar. A urine of high specific gravity with decreased quantity is frequently met with, which usually contains an excess of mucus, urates and coloring matter, but no sugar. Such urines will often reduce alkaline copper solutions and the alkaline bismuth solution, but if subjected to the preliminary treatment with $CuSO_4$ or with $Pb(C_2H_3O_2)_2$ mentioned under test No. 1, no sugar will be found.

Glycuronic Acid, $C_6H_{10}O_7$, or $CHO(CH.OH)_4\ CO.OH$, is a derivative of glucose found in the urine in combination with certain alcohols and phenols. It may be increased by the administration of certain remedial agents, such as chloral, chloroform, phenol, cresol, thymol, guaiacol, hydrochinon, resorcin, orcin, turpentine, camphor, acetanilid, kairin, morphin and from the production of certain aromatic substances as indol, phenol, etc. The acid occurs seldom or only in traces in the free state, but as glucosides of the aromatic nuclei derived from the above aromatic bodies. By heating the urine with dilute acids these glucosides are decomposed and the free acid is formed. The chief interest of glycuronates is their property of reducing alkaline copper, indigo, and bismuth solutions in testing for sugar. They give a positive reaction, like the pentoses, with HCl and phloroglucin, give the furfural reaction, and give crystalline compounds with phenyl hydrazin, but do not undergo fermentation with yeast.

It is partially precipitated by lead acetate, and by heating with $CuSO_4$ or $Cu(C_2H_3O_2)_2$ in faintly acid solutions.

Beta-Oxybutyric Acid, Diacetic Acid and Acetone.—These related substances are sometimes found in urine. *Acetone* is found in traces in normal urine and is most abundant when little or no carbohydrates are taken, and during starvation. Acetone is therefore believed to be derived from the proteids of the tissues and food.

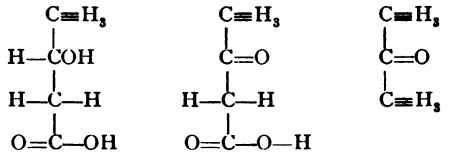
In diseased conditions acetonuria is most abundant in the specific fevers, as typhoid, pneumonia, scarlatina, measles, acute miliary tuberculosis, acute articular rheumatism, diabetes mellitus, septicemia, carcinoma of the stomach and in paresis.

It is found after chloroform narcosis, and often in the intoxications of intestinal origin.

Diacetic Acid, or Aceto-acetic Acid in the urine is to be regarded as pathological. It is found in most diseases in which acetone is present, and has the same significance. It is met with in various digestive and nutritive disorders, the digestive fevers of children, and is of especial significance in diabetes.

Beta-Oxybutyric Acid is met with in diabetic urines and especially in the severe cases. It occurs in combination chiefly as an ammonium salt. This acid is regarded as an antecedent of diacetic acid and acetone, and when it is present in the urine it is usually associated with these substances.

The relation of these substances is seen by comparing their graphic formulæ:



Oxybutyric Acid. Diacetic Acid. Acetone.

Oxybutyric acid, by oxidation outside of the body, yields diacetic acid. Diacetic acid readily breaks up into acetone and a carbonate, in alkaline solutions, and it is believed that these changes take place in the body.

In most of the specific fevers, and especially in certain digestive diseases, and in diabetes mellitus, there is a condition of decreased alkalescence of the blood, known as acid-intoxication. The chief acid causing this condition is oxybutyric acid, and to this acid intoxication the production of diabetic coma is quite generally attributed.

The significance of these compounds in the urine of diabetics is therefore apparent.

An increase in the amount of acetone, diacetic acid, or oxybutyric acid, in the urine of diabetics, is usually indicative of an unfavorable prognosis and the approach of coma. An estimation of these organic acids in a case of diabetes is therefore of more importance in indicating the prognosis than the estimation of the sugar.

The Detection of Acetone. Legal's Test. — Four or five c.c. of the urine are treated with a few drops of a freshly made solution of sodium nitroprusside, and then with a strong solution of NH_4OH. The red color produced, which appears in from five to ten minutes if acetone be present, gives place to a purple or violet color on the addition of acetic acid.

For a more accurate test it is necessary to distil the urine, and apply this or the following test to the distillate:

Lieben's Test. — To several cubic centimeters of the distillate add a few drops of a solution of iodine in potassium iodide, and then a solution of KOH. If merely a trace of acetone be present, a precipitate of iodoform crystals is deposited. This test is reliable and delicate in the absence of lactic acid and alcohol, but if NH_4OH be used for the KOH, alcohol will not form iodoform, while the acetone will do so.

Denniges' Test. *Reagent.* — Twenty grms. H_2SO_4, are poured into 100 c.c. of distilled water, and 5 grms. of freshly prepared yellow oxide of mercury added. After standing 24 hours it is ready for use.

To about 5 c.c. of urine add the reagent drop by drop, until a permanent precipitate remains after agitation, and then add a few more drops.

Filter, and add to the clear filtrate 3 c.c. of the reagent and 4 or 5 c.c. of a 30 per cent. solution of H_2SO_4, and boil for a minute, or better, set in a beaker of boiling water.

In presence of acetone or diacetic acid in appreciable amounts a heavy white precipitate immediately forms. If but traces are present, a slight cloudiness develops on standing a few minutes. When albumin is present a larger amount of the reagent must be added before boiling. This test is delicate and does not require distillation of the urine.

Diacetic Acid usually occurs together with acetone, and in the presence of ferric chloride produces a wine-red color, which is not entirely characteristic, because other substances produce the same color. The following process will serve for its detection: A fairly strong solution of ferric chloride is cautiously added to the urine, and if a phosphate precipitates, this is removed by filtration, and more Fe_2Cl_6 added to the filtrate. If a red color appears, it is divided into two portions. One portion is boiled, whilst the other is treated with H_2SO_4, and extracted by shaking with ether. Urine that

has been boiled does not give the red color with Fe_2Cl_6, or it loses its red color on boiling. This serves to distinguish this acid from phenol, salicylic acid, etc., which give similar colors with ferric chloride. After treatment with H_2SO_4 and shaking with ether, the aceto-acetic acid will be found in the ether. The ether may now be evaporated, and the residue treated with Fe_2Cl_6 solution, when the violet-red color will be obtained if it be present. The urine for this test must be fresh, as diacetic acid is rapidly converted into acetone on standing. Acetone is an oxidation product of diacetic acid.

Hydroxybutyric acid is found in the blood of diabetic patients, and it has been found in the urine in scarlatina, measles, in scorbutus and in starvation. It gives the same color-reaction with Fe_2Cl_6 as diacetic acid. It is best to separate it from the urine with ether, as above described for diacetic acid, before applying the test. Oxybutyric acid may be detected by evaporating a portion of the urine on a water-bath to a syrupy consistency, and distilling with an equal volume of concentrated H_2SO_4. The acid is thus changed into alpha-crotonic acid which distils over.

$$\underset{\text{Oxybutyric acid.}}{CH_2{-}CH.OH{-}CH_2{-}CO.OH} - H_2O = \underset{\text{Crotonic acid.}}{CH{-}CH{-}CH_2{-}CO.OH}.$$

By cooling the distillate, the crotonic acid crystallizes when it may be filtered out. Or, the distillate may be shaken with ether, and the acid be obtained on evaporating the ether. One should expect to find oxybutyric acid only in urines containing also diacetic acid.

An approximate estimation of the oxybutyric acid, or of the amount of *acid intoxication*, may be made by an estimation of the ammonia in the freshly voided urine. Free acid can not exist in the blood; bases must be found to saturate the acids, and in acid intoxication the blood being unable to spare the fixed alkalies, ammonia, which is always a product of retrograde tissue metabolism, is used to saturate the acids. The acids are eliminated therefore as ammonium salts. The estimation of ammonia thus becomes important.

Estimation of Ammonia. — To 25 c.c. of the fresh urine, in an evaporating dish or other shallow vessel, add 10 c.c. of milk of lime. Upon this vessel, supported by a triangle made of glass tubing or rod, place a smaller vessel, containing exactly 20 c.c. of $\frac{1}{4}N$ H_2SO_4. Place the dish upon a ground-glass plate, and cover with a tightly fitting bell jar, and let set three to four days, when the ammonia will have been absorbed by the acid. The acid is then colored with methyl orange or cochineal, and the free acid titrated with a $\frac{1}{10}N$ NaOH.

The 20 c.c. of the original acid would have required 50 c.c. of $\frac{1}{10}N$ NaOH. Each c.c. of the NaOH solution less than 50, required after the absorption of the NH_3, represents 0.0017 grms. of NH_3. It has been estimated that a daily output of 2 grms. of NH_3 corresponds to about 6 grms. of the organic acid, 5 grms. of NH_3 to about 20 grms. and 8 grms. of NH_3 to about 35 to 40 grms. More than 5 grms., of NH_3 occurring in the daily urine of a diabetic indicates danger of the approach of coma.

Lipaciduria is a term which has been applied to the condition in which volatile fatty acids are found in the urine. These occur normally in traces, especially formic,

acetic and butyric acids. As a symptom of disease, however, they have no significance. Formic, acetic, propionic, and butyric acids have occasionally been detected in the urine of fevers, in certain diseases of the liver, and in diabetes. For their detection the urine is distilled with phosphoric acid, and the test applied to the distillate. For simpler tests we may apply the following: Acetic acid may be detected by the odor of acetic ether when the distillate is warmed with alcohol and sulphuric acid. Ferric chloride gives a red tint, which disappears on boiling if acetic acid is present. Formic acid gives a white precipitate with silver nitrate, which blackens on warming.

Fat. Chyluria. — Fat occasionally occurs in the urine and gives to it a more or less turbid appearance, which clears on shaking the solution with ether. On separating and evaporating the ether, the fat remains behind. In chyluria the opacity is due both to the fat and to albuminous substances in imperfect solution. In some cases the appearance of this turbidity is intermittent, appearing only at certain times of the day; in others it is constant. In some cases chylous urine deposits a spontaneous clot of fibrin, while in others it does not. The fat may be separated by extraction with ether, but the turbidity still remains. In some rare cases, however, the turbidity disappears with the extraction of the fat.

Detection. — Its detection is usually sufficiently easy from the milky-white color and the separation of the fat on standing. Microscopically, the fat globules can be detected in some cases, but in others the microscope fails to reveal them. The author has seen a case where they were not visible with a $\frac{1}{16}$-inch objective.

Bile. — Urine containing bile usually has an abnormal color — either a brilliant yellow, a greenish-yellow, or brown. When the bile is abundantly present, the froth or foam produced on shaking the urine is quite permanent and is more or less colored. A piece of filter-paper or linen moistened with such urine retains the yellow color on drying.

Gmelin's Test. — Upon 1 or 2 c.c. of a partially decomposed yellow nitric acid, in a test-tube, carefully float 4 or 5 c.c. of the suspected urine. If bile coloring-matters be present, a succession of colors will appear in the urine, beginning with green, then passing through blue, violet, red and yellow, the green appearing at the top and the others below in the order named. The green color is always present when bile is present, but the reddish-violet color must not be taken for evidence of bile, as the normal coloring matters of the urine may produce such a coloration.

If the decomposed nitric acid, or nitrous acid, be not at hand, it may readily be prepared by adding a fragment of zinc to ordinary pure nitric acid. This test may also be applied as follows: The urine may be mixed with a concentrated solution of sodium nitrate, and the

mixture floated upon sulphuric acid, when the play of colors will be obtained as before; or, a crystal of sodium nitrate may be dropped into strong sulphuric acid and the urine floated upon this.

The Author's Test. — To 5 c.c. of urine add about half its volume of strong HCl and a few drops of Fe_2Cl solution and mix well by agitation.

In the presence of bile pigments the mixture assumes an emerald green color. It is necessary to remember that indoxyl gives a blue color under these conditions, which is easily dissolved and separated with chloroform, which does not dissolve the green substance.

Marechalt's Tincture of Iodine Test. — Upon the surface of the urine in a test-tube, float a few drops of tincture of iodine. Just below the line of contact of the two fluids there appears, after a few minutes, a beautiful emerald-green zone when biliary coloring-matters are present. This test seems to be delicate and reliable.[1]

Biliary Acids. — While the acids usually occur in the urine of jaundiced patients, along with the coloring matters, their detection is not so easy. We may use the following test: Evaporate the liquid to dryness and treat the residue with alcohol and filter. After evaporating the alcohol, apply **Pettenkofer's test** to a solution of the residue in water, as follows: To the solution of the biliary acids or their salts, add a few drops of cane-sugar solution (1:10) and then strong H_2SO_4. A cherry-red followed by a purple-violet color indicates biliary acids. **Oliver's peptone test** is, however, applicable to urine. The reagent is prepared as follows: Pulverized peptone, 2 grms.; salicylic acid, 0.250 grm.; acetic acid, 2 c.c.; distilled water sufficient to make 250 c.c. The urine, rendered perfectly clear by filtration, is rendered acid and diluted until the specific gravity is 1008. One c.c. of this diluted urine is run into about 4 c.c. of the above test solution. If biliary salts are present, a distinct milkiness promptly appears, but it becomes more intense in five minutes. Albumin, if present, should be separated before the application of this test. The test is very delicate and apparently reliable.

Pigments and Chromogens. — Eleven varieties of coloring-matters have been mentioned by various chemists as occurring in human urine, viz.: Urobilin, urochrome, urospectrin, uroerythrin, indirubin, indican, uromelanin, urorosein, urian, omicholin, omicholic acid. The following six chromogens have been described: reduced urobilin, urochromogen, uroroseinogen, indicanogen, indirubinogen and urocyanin.

[1] Jolles says that antipyrin in urine gives a similar green color.

It is believed by Gautrelet, as a result of a recent exhaustive examination, that normal urine contains but two primary pigments and that the others are derivatives of these two. These are *urobilin* and *uroerythrin*. He admits one transitory pigment, *urochrome*, and four chromogens or bodies which can easily be converted into pigments. These are urobilinogen, urochrom-erythro-roseinogen, indigogen and indirubinogen. The whole subject is complicated and unsettled.

Urobilin or *urochrome*, is the pigment to which urine owes its amber color. It is probably derived from bilirubin, which is derived from hemoglobin. It is *increased* in all diseases where there is an increased destruction of red blood corpuscles and during the resorption of large blood clots.

It is diminished in some forms of anæmia, chronic parenchymatous nephritis, diabetes, certain nervous diseases and diseases of the bone marrow. Like pathological urobilin, it gives a green fluorescence on the addition of ammonium hydroxide and a few drops of a strong solution of zinc chloride.

Uroerythrin is the pigment which gives the red color to uric acid and uratic deposits in the urine. It is *increased* in acute febrile diseases, such as pneumonia, influenza, typhoid fever, malaria, acute diseases of the liver in which the function of the hepatic cells are interfered with, acute articular rheumatism and in chronic diseases of the heart and lungs. When increased in amount it is usually accompanied by urobilin. When increased, the urate deposits are highly colored. It is precipitated by acetate of lead and barium chloride solutions, coloring the precipitates caused by these reagents. From the depth of color, its amount may be approximated.

Pathological Pigments. — Hemoglobin has already been spoken of. Hematin is only rarely seen. It is best detected by making the urine strongly alkaline with NH_4OH, filtering and examining the filtrate with the spectroscope, when the appearance seen in No. 12 (Frontispiece) will be seen. The reduced hematin may also be examined (see No. 13).

Hematoporphyrin, $C_{16}H_{18}N_2O_3$, is a pigment occasionally found in the urine of cases of rheumatism, Addison's disease, pericarditis, hepatic cirrhosis and croupous pneumonia. It is more frequently seen in the urine of persons taking sulphonal, trional or tetronal for some time and also in intestinal hemorrhage and lead poisoning. When present in considerable amount the urine has a reddish or port-wine color.

It is best identified with the spectroscope.

The urine is precipitated with BaCl, the precipitate filtered out, washed with water, then absolute alcohol, and rubbed up in a mortar with ordinary alcohol, acidulated with HCl and again filtered. The solution will have a red color if hematoporphyrin is present, which gives with the spectroscope four absorption bands, two broad and dark and two narrow and light.

Pathological Urobilin. — This pigment is often spoken of as *febrile urobilin* from its frequent occurrence in febrile urines. It is said to be identical with stercobilin of the fæces.

It occurs in certain hepatic diseases, hepatic cirrhosis, chronic malaria, pernicious anæmia, carcinoma, scorbutus, Addison's disease, extra-uterine pregnancy, after cerebral hemorrhages, hemophilia and in most infectious fevers such as measles, scarlatina, typhoid fever, etc.

Detection. — Urobilin is best detected by the spectroscope. 1. Acidulate 10 to 20 c.c. of the suspected urine with a few drops of HCl, and then shake with 6 to 10 c.c. of amyl-alcohol. Allow the alcohol to separate and examine the clear alcoholic layer with the spectroscope. (See Spectrum No. 16, p. 32.)

2. To a portion of the amyl-alcohol solution, drawn off with a pipette, add a few drops of a one per cent. solution of zinc chloride in alcohol, which has been made strongly alkaline with NH_2OH. A beautiful green fluorescence appears if urobilin is present and the solution gives the spectrum of alkaline urobilin. (See Spectrum No. 17.)

3. Urines which are rich in urobilin usually have a dark-yellow color, resembling biliary urine. The foam of such urine is also colored.

Ten to 20 c.c. of the suspected urine may be shaken well with 10 c.c. of chloroform. The chloroform is separated and shaken with a few drops of a dilute solution of iodine in potassium iodide, and then with a few c.c. of a dilute solution of NaOH. The chloroformic solution, in presence of febrile urobilin, assumes a yellowish-brown color and shows the green fluorescence.

The Chief Chromogens found in normal urine are *urohematin, uroroseinogen, indoxyl* and *skatoxyl.*

Urohematin is a chromogen which yields a red color on treatment with strong HCl. It has the same significance as indoxyl.

Uroroseinogen. — This is another chromogen which yields a rose-red color when treated with mineral acids. The rose-red or deep red ring often seen at the contact zone in applying Heller's nitric acid test for albumin is due to the presence of this chromogen. (**Rosenbach's reaction.**) If to 5 c.c. of urine 1 c.c. of HCl be added, a rose-red color appears in about 10 minutes, if much urobilin be present. The coloring matter is removed by shaking with amyl-alcohol.

Indoxyl. — This chromogen is found in traces in normal urine. It is pathological only when in increased amount. Its origin is the indol, C_8H_7N, produced in the intestinal canal during the digestion of proteids. This is absorbed, oxidized in passing through the blood to indoxyl, C_8H_7NO, and then combines with H_2SO_4 or glycuronic acid, and is eliminated in the urine as **sodium** or **potassium indoxyl-sulphate** or **indoxyl-glycuronic acid** or **indican.**

$$C_8H_7NO + H_2SO_4 = \begin{matrix} C_8H_6NO \\ HO \end{matrix}\!\!\rangle SO_2 + H_2O.$$

Indoxyl. Indoxyl Sulphuric Acid.

$$\underset{\text{Indoxyl Sulphuric Acid.}}{C_8H_6NO\diagdown\!\!\diagup HO}SO_2 + \underset{\text{Sod. Phosphate.}}{Na_2HPO_4} = \underset{\text{Indican.}}{C_8H_6NO\diagdown\!\!\diagup NaO}SO_2 + \underset{\text{Acid Sodium Phosphate.}}{NaH_2PO_4}.$$

When indican is treated with strong HCl, it is decomposed into indoxyl and either glycuronic or sulphuric acid and sodium or potassium chloride. If now an oxidizing agent be added to the solution, such as nitric acid, the hypochlorites, the nitrites or ferric chloride, the indoxyl is converted into indigo blue.

$$2C_8H_7NO + O_2 = C_{16}H_{10}N_2O_2 + 2H_2O.$$

Indigo-blue is sometimes formed spontaneously, either before or after voiding the urine, and may appear as a sediment.

Detection. — To 10 c.c. of the urine add an equal volume of concentrated HCl, containing 0.5 per cent. of Fe_2Cl_6. Or, after adding pure HCl, we may add about three drops of the ordinary Fe_2Cl_6 solution. On standing a few minutes, a dark cloud will be seen near the top of the mixture. On mixing the solutions, and adding about 2 c.c. of chloroform, the indigo is dissolved by the chloroform, and appears as a blue liquid at the bottom of the tube. From the depth of the blue color, we may judge of the amount of indican present.

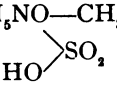
Jaffe uses two to three drops of a weak solution of sodium or calcium hypochlorite in place of the Fe_2Cl_6. When the hypochlorites are used great care must be taken not to add enough to bleach the indigo-blue. When this precaution is used, the reaction is more marked than with Fe_2Cl_6. When the urine is dark in color, and when it contains bile, it may be partially decolorized by adding a small quantity of lead subacetate solution, taking care not to add an excess. The precipitate is filtered out and the test applied to the filtrate. Some indigo red is usually formed at the same time as the indigo-blue.

This red coloring matter is not soluble in chloroform but is soluble in ether. Some believe it is identical with the red pigment obtained from urohematin.

Skatoxyl. — In putrid decomposition of proteid matters in the intestines both indol and skatol are produced. They are both oxidized and appear together in the urine.

$$\underset{\text{Skatol.}}{C_8H_6N\text{–}CH_3} + O = \underset{\text{Skatoxyl.}}{C_8H_6NOCH_3}$$

The skatoxyl combines with H_2SO_4 to form skatoxyl sulphuric acid,



When urine contains a large amount of skatoxyl, the urine grows darker on standing exposed to the air, beginning at the surface and gradually passing downward. The color is not a brown-red, but a reddish-violet. This change takes place more rapidly when strong HCl is added, and immediately when nitric acid is added. This is known as the *Rosenbach reaction.* The addition of oxidizing agents is not needed to develop the red color, as in the case of indoxyl. This red pigment is insoluble in chloroform or ether, but may be easily separated with amyl-alcohol.

Clinical Significance. — The significance of indican and of skatoxyl is the same. As they are produced by secondary fermentations of the proteid contents of the intestine, chiefly the colon, the amount of the pigments found in a given urine may be taken as a measure of the bacterial putrefaction of proteids in the intestinal canal.

When the peristaltic movement of the small intestine is diminished, as from fright, excessive brain work or worry, neurasthenia, ileus and peritonitis we may expect to find an increased output of indican.

In simple constipation, which is usually due to a storage of the contents in the large intestine, where the liquid portion is soon absorbed, thus checking putrefactive changes, indicanuria is usually absent. In deficient secretion of HCl in the stomach, as in gastritis and gastric catarrh, carcinoma, involving a large area of the stomach, gastric atony, and at the height of most acute fevers, we may expect an increase of indican.

Indican is also found in the urine of persons suffering with empyema, putrid bronchitis, gangrene of the lung, or in other suppurative conditions, where pus is undergoing putrid decomposition. When such decomposition of proteid matters in other organs can be eliminated, an excess of indican means intestinal proteid decomposition. It must be remembered, however, that a diet very rich in the so-called "red meats" will favor the production of indol in the intestine, and indicanuria.

Melanogen and Melanin. — Some urines on standing turn dark brown in color. Such urines usually contain melanogen, which on exposure to air oxidizes to melanin. This chromogen is usually associated with the existence of melanotic tumors.

Melanogen may be detected by treatment with bromine-water, with which it forms a precipitate, which is yellow at first but gradually turns black.

Ehrlich's Diazo-reaction. — Under certain pathological conditions, the urine contains a chromogen which gives a deep cherry-red color with diazo-benzene-sulphonic acid and ammonia. This reaction is generally known as the diazo-reaction, first discovered and studied by Ehrlich. This reaction is obtained in the urine of persons suffering from certain specific fevers, especially typhoid fever, measles, septicemia and in some cases of phthisis.

Two solutions are prepared and kept in well-stoppered bottles, preferably in the dark: No. 1, one grm. of sulphanilic acid is dissolved in a mixture of 100 c.c. of water and 5 c.c. of hydrochloric acid. A second solution is made by dissolving 0.5 grm. of sodium nitrite in 100 c.c. of water. Five c.c. of urine are mixed with an

equal volume of mixture No. 1, and then 3 to 5 drops of the sodium nitrite solution. The solutions are well mixed and about 1 c.c. of NH_4OH solution is poured carefully down the side of the tube upon the mixture. Normal urine shows with this test a yellow or orange color, and a precipitation of phosphates. In certain of the above-named diseases, especially in typhoid, the urine gradually assumes a carmine-red color. The froth produced on agitation is also distinctly red, and the precipitated phosphates show a green or violet color. Many phenol derivatives give a similar color-reaction with the above test, and may lead to erroneous conclusions. According to Ehrlich, this reaction is characteristic of the urine in typhoid, measles, and acute tuberculosis. Others deny the value of the test, the difference being possibly due to the interference of phenol derivatives.

Ferments Found in the Urine. — Pepsin, trypsin and a diastasic ferment have been found in the urine in addition to the organized ferments of lactic, butyric and acetic acids and urea. The pepsin ferment of the urine is said to be absent in the urine of typhoid fever, carcinoma of the stomach, and, according to some, in nephritis.

Detection. — Pepsin is best detected by Sahli's method. A little pure fibrin is placed in the urine and set aside for several hours. It is then removed, placed in diluted HCl (0.2 per cent.), and the mixture kept at a temperature of from 30° to 40° C. (86° to 104° F.). Any pepsin present in the urine is taken up by the fibrin, and the latter is slowly digested in the acid fluid.

The diastasic ferment is detected in the usual manner, by its effect upon starch-mucilage. The milk-curdling ferment has occasionally been found in the urine.

Ptomains, or **diamins,** have been found in healthy urines as well as in morbid urines. In most fevers, especially in the specific and contagious fevers, the urine contains certain poisonous alkaloids. These can be detected by first acidifying the urine and filtering from any mucus present, and then precipitating with the double iodide of potassium and mercury. The precipitate, which contains these bases, is distinguished from albumin or other substances by its solubility in alcohol at a gentle heat.

The **diamins** of the urine may be precipitated as benzoyl compounds by benzoyl chlorid, and caustic potash. By this means cadaverin, putrescin and other diamins have been detected in the urine of vesical catarrh. Normal urine is said to be free from these bodies.

Estimation of Renal Permeability. Mering's Phloridzin Method. — The functional power of the renal cells may be determined by the subcutaneous injection of 0.005 grm. (one twelfth grain) of phloridzin, which has the effect of stimulating the renal cells, under which they separate sugar from the blood. There is thus produced a temporary glycosuria. Normal healthy cells will eliminate more sugar in a given time than diseased cells. The elimination of sugar by normal cells begins in about ten to fifteen minutes after the injection, while it begins later when the renal cells are degenerated. In normal conditions it reaches its height in thirty minutes and lasts from one to two hours. By an estimation of the quantity of sugar excreted, every half hour for two hours, we may determine the *secreting power or permeability* of the renal cells.

The Methylene-Blue Method. — This method consists in injecting subcutaneously 0.020 grm. (one third grain) of methylene-blue and observing how soon it appears in the urine and how long it continues to

color it. This method is not so certain as the phloridzin method. The greatest use of these methods is in conjunction with catheterism of the ureters, by which the urine may be collected from each kidney separately. When the two methods are applied together it is possible to detect a one sided nephritis, or other disease of one kidney.

It is of the utmost importance when it is proposed to remove a kidney, to test the permeability of the other one. This fact may usually be determined by an estimation of the total nitrogen or the total chlorine, eliminated by each kidney in an equal time. In normal kidneys the urine of both contains the same amount of nitrogen, chlorides, phosphates, and has the same specific gravity, except when tumors interfere with the local circulation.

The Cryoscopic Method. — Cryoscopy is the name given to the process of determining the depression of the freezing point of solutions. This method is used to determine the molecular concentration of the urine, or the renal permeability, by the freezing point of this secretion.

The process is based upon the following facts:

The freezing point of a solution of a substance in a liquid with which it forms no chemical union, is lowered below that of the solvent, in direct proportion to the number of molecules of dissolved substance in a given volume of the solution or its molecular concentration.

This law applies to a solution of several substances in the same solvent, as well as to solutions of one substance. The depression of the freezing point of aqueous solutions is, therefore, a measure of the number of molecules in that solution.

The number of these mixed molecules in an appreciable volume of urine, say 1 c.c., is very great. For present purposes the relative number is all that is needed. The simplest form of apparatus for determining the freezing point of urine is that of Claude and Balthazard (Fig. 10). It consists of a tube "*a*," the freezing vessel and "*b*," an outer tube to protect "*a*." The space between these two tubes is partly filled with alcohol to serve as a conductor. The cylinder *A* is

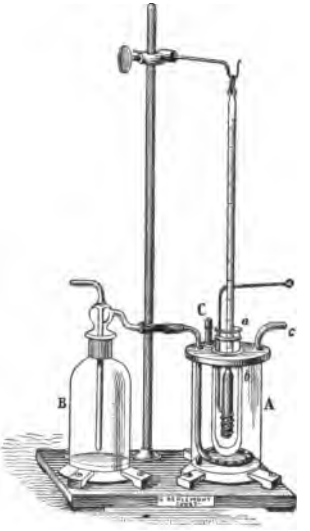


FIG. 10.

filled three fourths full with ether or carbon disulphide. The wash bottle *B* contains a small quantity of H_2SO_4 to dry the incoming air. The tube *c* is connected with a water aspirating pump, and a current of air drawn through the apparatus, which escapes from numerous small holes in the metal inlet tube, coiled on the bottom of the cylinder *A*, causing the ether to evaporate rapidly, thus producing a low temperature. The temperature can be regulated, or kept constant, by regulating the current of air. The liquid, whose freezing point is desired, is placed in the tube "*a*," ether or carbon disulphide is put in *A*, the vessel made tight, the thermometer, which should read hundredths of a degree, is suspended in the liquid to be frozen, with the platinum wire stirrer adjusted as seen in the cut, and the pump started.

From time to time the stirrer is moved up and down, until the thermometer becomes stationary at a little below 0°C. To cause the solution to freeze, scrape off a little frost from the outside of *A* and add it to *a*. It is not necessary to wait until the liquid is all frozen, but until a part is congealed. The temperature at which normal urine freezes is usually —1.30° C. to —2.20° C. The extremes are seldom met in health.

The depression of the freezing point is usually represented by Δ. The depression of the freezing point, expressed in hundredths of degrees, may be *arbitrarily* used to represent the relative number of molecules dissolved in a given volume, say 1 c.c. If we represent the number of c.c. of urine voided in 24 hours by *V*, then the total work done by the kidneys in 24 hours will be represented by $V \times \Delta$. To make more accurate the comparison in different individuals, we must take into the calculation the body weight of the individual. If we designate this weight in pounds or kilogrammes (2.2 lbs.) by *P*, the total daily work per lb. or kilo. will be represented by

$$\Delta \times \frac{V}{P}.$$

Example. — A certain urine gave a depression of freezing point, (Δ), of 1.20° C. The volume of urine was 1,200 c.c. The weight was 60 kilogrammes (132 lbs.). The work of the kidneys in this case was $120 \times 1{,}200 \div 60 = 2{,}400$ for each kilo. of body weight. Or, each kilo. gave 2,400 arbitrary molecules of urinary solids in 24 hours.

In health this number should be between 3,000 and 4,000. In pathological conditions the number is above or below these limits. A part of these molecules are inorganic, chiefly NaCl, filtered through the glomeruli with the water, and the rest organic, separated from the

blood by the tubular epithelial cells. According to the theory of Ludwig, as modified by Koranyi, there filters through the glomeruli nearly a pure solution of NaCl, containing too small a quantity of the other inorganic salts to appreciably affect the freezing point. This solution is concentrated in the tubules, by the absorption of a part of the water, and by an exchange between this solution and the blood serum of a certain number of NaCl molecules for an equal number of organic molecules. A determination of the relative number of molecules of NaCl, and of organic molecules, will enable us to measure the part played by the parenchyma or glomeruli, and that played by the tubular epithelial cells, in the formation of the urine in question. Sodium chloride is easily determined by titration. (See p. 56.) One per cent. of NaCl (10 grms. per liter), is known to depress the freezing point 0.605° C. The per cent. of NaCl multiplied by 0.605 will give the amount of depression caused by the NaCl present, and the remainder of the observed depression will be due to the organic molecules. We can thus determine the relative number of molecules separated by the glomeruli and the tubular epithelium. The latter is often designated by the sign δ.

Example. — A certain urine gave a freezing point of — 1.20° C. The titration of the NaCl gave 1.5 per cent. The quantity of urine in 24 hours was 1,200 c.c. and the body weight was 60 kilos. Then

$$\frac{.605 \times 1.5 \times 1{,}200}{60} = 1{,}800 \text{ arbitrary molecules of NaCl.}$$

The total number of molecules was

$$\frac{120 \times 1{,}200}{60} = 2{,}400.$$

The organic molecules were found by deducting 1,800 from 2,400, leaving 600 molecules as those elaborated by the epithelial cells of the tubules.

These two formulæ,

$$\frac{\Delta \times V}{P},$$

or the expression of the *total molecular diuresis*, and

$$\delta \times \frac{V}{P},$$

or the expression of the molecular exchange or activity of the tubular epithelium, are two important working formulæ.

The quotient obtained by dividing the total depression by that due to the elaborated (organic) molecules, Δ/δ is also of use. This quotient in normal urines is from 1.5 to 1.7 and not outside of these limits. When

$$\frac{\Delta V}{P} = 3{,}000, \frac{\Delta}{\delta} = 1.5$$

and when

$$\frac{\Delta V}{P} = 4{,}000, \frac{\Delta}{\delta} = 1.7.$$

In nephritis this last ratio may sink to 1.1, and in cardiac insufficiency it may rise to 2.5. This factor is often of great use in showing degeneration of the heart muscle, even when no abnormal heart sounds can be heard with the stethoscope.

Cryoscopy of the urine is sometimes of great value in the diagnosis of both renal and cardiac disorders, as well as certain disorders of metabolism.

We may state the chief uses of cryoscopy of the urine as follows:

1. To determine cardiac sufficiency, or insufficiency, with certainty even when the stethoscope fails.

2. To determine which portion of the renal structure is functionally deficient, and the degree of that deficiency.

3. To prognosticate uraemic accidents with exactness.

4. In conjunction with catheterization of the ureters, we may determine whether one or both kidneys are the subject of disease, and the relative functional power of each.

5. By it the fact has been established that functional permeability of the kidneys in most forms of nephritis is intermittent. There are periods of entire permeability followed by periods of reduced permeability, and consequent danger of uremia.

6. When one kidney is to be removed, for any cause, a cryoscopic examination of the other one should be made to determine its condition, unless this can be done by the phloridzin or methylene blue method. Cryoscopy is more certain and more quickly done.

MICROSCOPICAL EXAMINATION OF SEDIMENTS.

A complete analysis of urine always includes a microscopical examination of any sediment it may contain. Indeed, we should regard such an examination as of more value, from a diagnostic point of view,

than the chemical analysis. Without it an exact diagnosis of the various organic renal and vesical diseases is impossible.

Method of Obtaining the Sediment. — Three methods of obtaining sediments for examination are in common use, viz.: (1) *Sedimentation by gravity*, by allowing the urine to set from 6 to 12 hours in a cool place, and (2) *Centrifugal sedimentation*, obtained by the aid of a centrifugal machine. The latter method takes advantage of the difference in the specific gravity between the sediments and the liquid, as in the former, but hastens the separation by applying centrifugal force to aid gravity. (3) *Concentration of the sediment by filtration.* Each of these methods possesses certain advantages as well as disadvantages. The gravity method has the advantage of being less violent, and hence is not liable to break up delicate structures. It has the disadvantage of requiring time, and some danger of changes taking place in the deposits during the natural separation of the sediment. The centrifugal method requires but a few minutes, and can be done while the urine is fresh. It separates any bacteria present in large numbers, and is especially useful in the search for tubercle-bacilli and gonococci. It has the disadvantage, at times, of causing more or less change in pus cells, epithelial cells, spermatozoa and friable casts. While these changes are not great, the sediments may not appear exactly as they were when the urine was voided. This method has the advantage of enabling us to concentrate sediments in searching for hyaline casts, but it must be noted that mucus-threads, and the so-called cylindroids, closely resembling hyaline casts, will be more abundant in a sediment separated by centrifugal force than one separated from the same urine by gravity. They may easily be mistaken, and often are mistaken, for hyaline casts.

The third method of obtaining the sediment is conducted as follows: An ounce or two of the urine is poured upon a previously well-washed filter and the greater portion of the water allowed to filter through. Before the last few c.c. filter through, a portion may be drawn out with a pipette, transferred to a slide and examined at once. Or, the paper may be punctured with a needle and the last few c.c. received in a centrifuge tube, diluted with water, and separated by the centrifugal machine. This method saves waiting for the sediment to settle, and can be done in the usual routine filtering of the specimen before applying chemical tests. In the examination of alkaline urines, as in cases of chronic cystitis, it is very important that this should be done at the earliest possible moment after being voided, as the cellular elements rapidly dissolve in such urines, and change their whole character.

Magnifying Power. — A sediment should be transferred to the

slide, with a medicine dropper or a camel's-hair brush, the coverglass dropped on it, and examined first with a low power objective. A half-inch lens gives the best results, in affording sufficient magnifying power and enabling one to run over the whole field rapidly, first with good central illumination, then with a partially darkened field and oblique illumination. When an object is found which it is desired to examine more carefully a higher power is brought into use. A good one sixth in. objective is a convenient lens. A double nosepiece is an essential. An Abbé condenser is useful in the examination of bacteria, but it is not necessary for ordinary work. For the proper examination for bacteria, a dry one-eighth or a one-tenth inch objective will be needed.

The Use of Stains. — In the ordinary examination of sediments, stains are not necessary or advisable. Sometimes stains will aid in differentiating morphological elements. Knapp claims that the best stain for this purpose is sodium alizarinate. This dye stains the mucus in healthy urine red, while in nephritic patients, it either does not stain at all or it is colored a light yellow. Granular casts are stained yellow, hyaline casts light violet. The nuclei of the leucocytes in pyelitis are stained yellow, and in fresh urine of cystitis not at all.

P. Liebmann stains the centrifugalized sediment by adding to it from 2 to 4 drops of a solution consisting of 2 grms. of methylene blue to 100 c.c. of a 10 per cent. formalin solution. After well mixing the stain with the sediment, water is added to nearly fill the centrifuge tube, and the sediment is again separated by the centrifugal machine. The hyaline casts will be stained a light blue, waxy casts a deep blue, in granular casts, the granules will be deeply stained with a light blue background. The nuclei of epithelial and round cells and of the leucocytes will be deeply stained blue. Bacteria are also deeply stained.

Preservation of Urine and Sediments. — When specimens of urine are to be sent by mail for examination, or if the examination is deferred for any reason, it is often necessary to add some antiseptic to prevent decomposition. This is rarely necessary in concentrated, very acid urines, as the aromatic substances present in such urines will preserve them for some days or even weeks.

The antiseptics employed are chloroform, chloral, formalin, thymol, boric acid, chromic acid, potassium dichromate and in the following amounts:

Chloroform, three drops to the fluid ounce. This and chloral reduce Fehling's solution.

Chloral, 1 grm. (15.5 grs.) per fluid ounce.

Formalin, two drops per fluid ounce. Coagulates albumin if present.

Thymol, five drops of 1 per cent. solution in alcohol to the ounce. It gives a white zone in Heller's test for albumin, which is soluble in alcohol.

FIG. 11.

Chromic acid, two drops of a 2 to 5 per cent. solution to the ounce. It coagulates albumin.

Potassium dichromate, five drops of a 5 per cent. solution per fluid ounce.

Urinary Deposits or Sediments. — Normal urine is clear, but on standing it will usually deposit more or less sediment. Urine that is

turbid when passed will usually deposit a sediment, which may contain mucus, pus, blood, chyle, earthy phosphates, acid urate of sodium, or an abundance of epithelial cells from the kidney, ureters, or bladder. A turbidity which appears within a few hours after the urine is voided is most likely to be due to acid urates, the oxalate of lime, or the earthy phosphates. When such a deposit is to be examined, a few ounces of the urine are set aside in a cylinder or tall vessel for 12 hours to allow the sediment to accumulate. The sediment may be separated from the fresh urine by the **centrifugal machine.** The urine to be examined is well shaken and poured into specially constructed tubes (*C*, Fig. 12); these are placed in the receptacles (*A*) and revolved. The sediment forms at the bottom of the tube.

FIG. 12.—DEPOSIT IN "ACID FERMENTATION" OF URINE. (*a*) FUNGUS; (*b*) AMORPHOUS SODIUM URATE; (*c*) URIC ACID; (*d*) CALCIUM OXALATE.

This is more satisfactory in some respects than spontaneous sedimentation, because some sediments undergo changes on standing in contact with urine. The usual form of hand centrifuge is shown in Fig. 12. A great number of machines have come into the market in recent years. Of those intended to be driven by electricity that devised by Purdy is perhaps the most serviceable. For sedimenting urine a water-motor centrifuge is to be recommended where an electric light current is not available. It requires from three to four minutes to obtain a complete sedimentation of ordinary urine. The sediment may be removed from the solution by means of a pipette, or narrow glass tube, drawn down at one end. By holding the finger upon

the upper end until it is depressed to the bottom of the glass, and then, removing the finger for an instant, the sediment will be drawn up into the tube when it may be removed for examination. It is more convenient, for the microscopial examination of urinary sediments, to either **dispense with the usual cover-glass,** as a larger field is available for search for characteristic objects, or to use a coverglass about one inch in diameter. The chief **crystalline deposits** are uric acid, urates, calcium oxalate, the phosphates or carbonates of magnesium and calcium, cystin, hippuric acid, leucin, tyrosin, etc. **Organized deposits** include leucocytes, blood, pus, casts, epithelial cells, fungi, and bacteria.

The chemical examination of the deposits should be preceded by a microscopical examination. In fact with a little experience, the

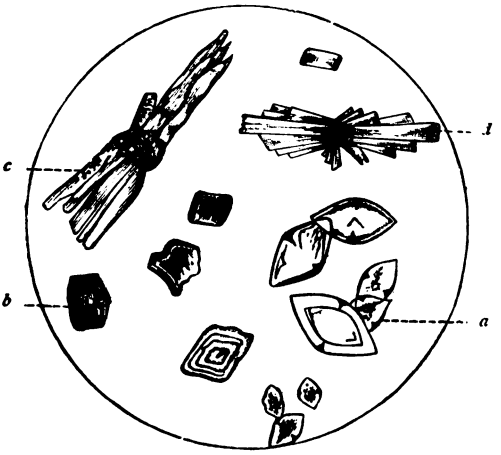


FIG. 13.—URIC ACID. (*a*) RHOMBIC TABLES (WHETSTONE FORM); (*b*) BARREL FORM; (*c*) SHEAVES; (*d*) ROSETTES OF WHETSTONE CRYSTALS.

microscopical examination usually renders the chemical examination entirely unnecessary, as most of the unorganized and crystalline sediments may be easily recognized by microscopical better than by chemical means.

Crystalline Deposits. — Uric acid occurs in crystals, differing much in form and size, and usually, though not always, stained a brownish-yellow to a light lemon-yellow color by uroerythrin. They are sometimes large, and when grouped together, as at *d*, Fig. 14, are large enough to be seen with the naked eye, and are commonly called

"gravel." They dissolve when warmed with NaOH solution. The most characteristic forms are those shown in Figs. 13 and 14. The appearance of uric acid as a sediment does not necessarily imply an

FIG. 14. — DEPOSIT OF AMMONIACAL URINE (ALKALINE FERMENTATION). (*a*) ACID AMMONIUM URATE; (*b*) AMMONIO-MAGNESIUM PHOSPHATE: (*c*) BACTERIA

increased elimination of this acid. It depends upon the degree of concentration of the urine, the reaction, and the temperature.

Acid Urates. — Amorphous urates consist principally of acid sodium

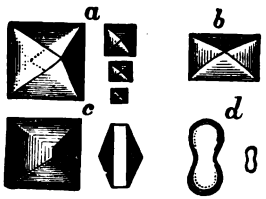


FIG. 15. — OXALATE OF LIME. (*a*) OCTAHEDRA; (*b*) BASAL PLANE OF AN OCTAHEDRON FORMING A RECTANGLE; (*c*) COMPOUND FORMS; (*d*) DUMB-BELLS.

FIG. 16. — PERFECT DUMB-BELL CRYSTALS OF OXALATE OF LIME.

urate. (See Fig. 12.) The deposit is amorphous unless a very high magnifying power is employed. Then it is seen to be made up of minute globular particles of yellow, red, or brown color. This sedi-

ment separates only from acid urines. It dissolves to a clear solution on adding a solution of NaOH or KOH, or when heated.

For the purpose of testing the solubility of the sediment under the microscope, it will be found convenient to place a drop or two of the solvent on the slide, at one side of the cover-glass, and put on the other side a piece of bibulous paper. In this way the fluid is drawn under the cover-glass on the one side and removed at the other, the old liquid being replaced by the new. In this way the action of reagents upon urinary sediments may be readily observed.

Acid sodium urate sometimes crystallizes during the acid fermentation, in the form of larger spheres made up of elongated crystals. They appear under the microscope as yellow or brown, frequently almost opaque spheres, with one or more spicules. When the urine becomes alkaline, from fermentation, the amorphous urate of sodium and free uric acid present are gradually converted into ammonium urate, which has the appearance seen in Fig. 14.

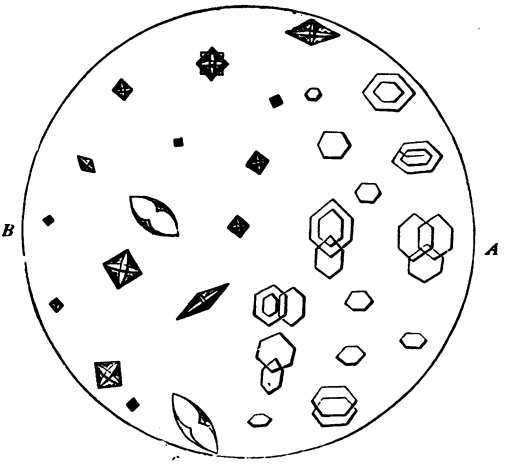


FIG. 17. — *A*, CRYSTALS OF CYSTIN; *B*, OXALATE OF LIME; (*c*) HOUR-GLASS FORMS OF *B*.

Calcium oxalate occurs as a sediment in transparent, strongly refracting, regular octahedrons, which are readily soluble in HCl, but insoluble in acetic acid. They frequently accompany uric acid crystals and deposit during the acid fermentation, as shown in figure 12. They are permanent in alkaline solutions and may therefore be found in either acid or alkaline urines. They are frequently called envel-

ope-shaped crystals, from the fancied resemblance to the reverse side of an envelope. They are usually of very small size, and occasionally appear in the form of dumb-bells (Figs. 15 and 16).

Clinical Significance. — A few isolated crystals of calcium oxalate have no clinical significance. They greatly increase after eating such vegetables as tomatoes, fresh beans, beet-root, asparagus, apples, grapes, honey, and after the administration of rhubarb, senna, squills, etc. Another source of oxalic acid in the body is incomplete oxidation of carbohydrates and proteid retrograde, decomposition products. For this reason it is frequently met with in excess in diabetes mellitus. It is frequently excessive in fermentative disturbances of the intestinal canal, and in certain nervous disturbances. The long-continued excretion of an excess of oxalate of

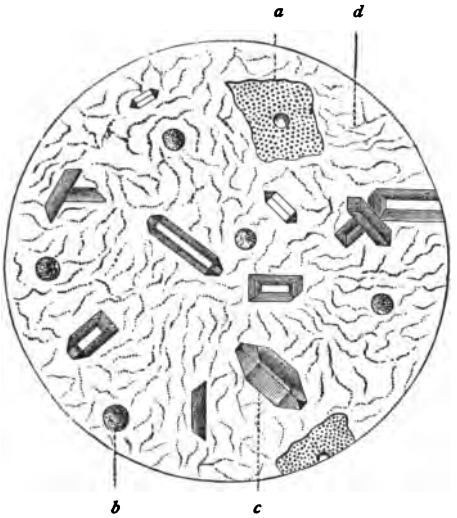


FIG. 18.—DEPOSIT FROM A CASE OF INFLAMED BLADDER (AMMONIACAL FERMENTATION). (*a*) DETACHED EPITHELIUM; (*b*) PUS-CORPUSCLES; (*c*) TRIPLE PHOSPHATE; (*d*) MICROCOCCUS UREÆ.

calcium frequently irritates the kidneys, producing albuminuria with hyaline casts, and grave nervous disturbance, and may lead to the formation of calculi, especially renal calculi. For this reason it is important to determine whether the crystals form in the kidneys or after the urine is secreted. This can usually be done by examining the fresh warm urine with the aid of the centrifuge. When the urine shows fairly large octahedral crystals or dumb-bells, under these conditions, there is danger of the formation of calculi. They are usually associated with an excessive amount of uric acid, nucleoalbumin, and phosphates, and sometimes albumin and casts.

Ammonium-magnesium phosphate (**triple phosphate**) occurs as a sediment in neutral or alkaline urine. The crystals are large, trans-

parent, highly refracting prisms, usually in the form seen in Fig. 19. Occasionally it occurs in the form of feathery crystals or star-shaped groups. They are never colored. They frequently attain a size sufficient to render them visible to the naked eye, especially in a strong light. To the naked eye the sediment of earthy phosphates appears as a bulky, opaque white sediment, rapidly settling to the bottom of the vessel. The sediment often looks like pus, but it is readily soluble in acetic acid, while pus and calcium oxalate are not soluble.

Magnesium phosphate is occasionally deposited in concentrated urines of feebly alkaline reaction.

Calcium phosphate occurs either as an amorphous or granular deposit of $Ca_3(PO_4)_2$ or as pointed, wedge-shaped prisms, either singly or in clusters. The crystals are said to be composed of the acid salt, $CaHPO_4$. Both forms are dissolved by acetic or hydrochloric acid.

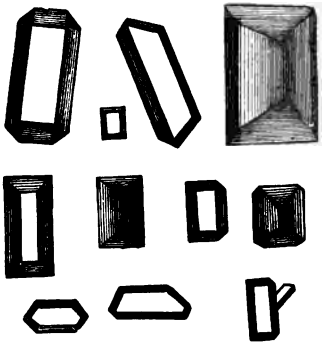


FIG. 19.—THE MORE USUAL FORMS OF TRIPLE PHOSPHATE. × 300.

Clinical Significance of Phosphatic Deposits.—The phosphates of calcium and magnesium are soluble in acid solutions and never occur in acid urines. They only appear in nearly, neutral or alkaline urines, and are easily dissolved by acetic acid. When phosphatic deposits occur in fresh urine it is pathological and indicates a disturbed metabolism, or fermentation of the urine in the bladder. This latter condition occurs in chronic cystitis, pyelitis, or retention. These conditions may be due to infection, calculus, new growths, enlarged prostate, or paralysis of the bladder, as in spinal disease, etc.

Calcium sulphate is rarely present as a urinary sediment. It occurs in the form of long, colorless needles or prisms, or in elongated tables with abrupt extremities.

Calcium carbonate occasionally occurs in the urine as an amor-

phous deposit, but on higher magnification it is seen to be made up of minute spherical granules. (See Fig. 22.)

Hippuric acid occasionally occurs as a sediment in the urine in the form of four-sided prisms, either occurring separately or in groups. They are soluble in ammonia, insoluble in HCl. It occurs especially after the administration of benzoic acid and after eating certain fruits, as cranberries, bilberries, etc. It is of no diagnostic importance.

Cystin.—The crystals of this body appear as regular hexagonal plates, superimposed or contiguous to one another. (See Fig. 17.)

FIG. 20.—HIPPURIC ACID.

They are insoluble in acetic acid, but soluble in ammonia. Cystin is sometimes also found in solution in the urine. It is a decomposition product of proteid matter, and is generally the result of bacterial action in the intestines. It is frequently associated with diamins and ethereal sulphates. It sometimes forms calculi.

Leucin and **tyrosin** usually occur together. Tyrosin occurs in the sediment in the form of sheaves of very fine crystals. **Leucin,** commonly associated with tyrosin, is more soluble than the latter, but occurs to some extent in the sediment in the form of small spheres, not unlike oil globules, which in a good light are seen to be marked with radiating striæ. When quite pure, leucin crystallizes in delicate plates, but as a urinary sediment it usually forms spheres. (See Fig. 21.) Tyrosin has been found in the urine, together with leucin, in

phosphorus poisoning, in acute yellow atrophy of the liver, in leukemia, and in some of the infectious diseases. Their presence, as a sediment, is usually accompanied by a low amount of urea.

Fat is deposited in the form of strongly refracting globules of varying size, and readily soluble in ether. It may be present in the urine in small quantities after the fracture of bones, and in some chronic cases of Bright's disease attended with fatty degeneration. In chyluria it occurs in greater abundance, although the droplets may be so small as to be observed only with high power objectives.

Indigo occasionally occurs as a sediment in concretions and amorphous fragments, and also in the form of blue crystals and clusters of

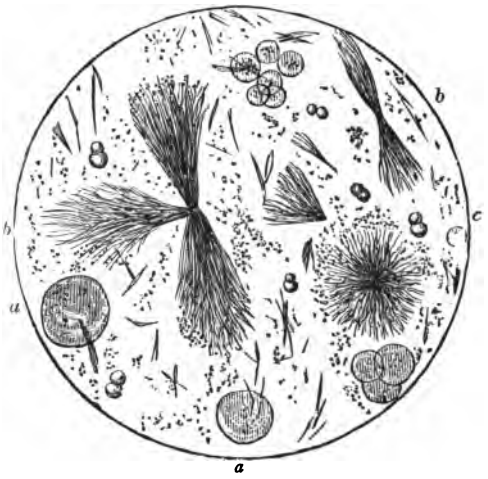


FIG. 21.—(*aa*) LEUCIN BALLS; (*bb*) TYROSIN SHEAVES; (*c*) DOUBLE BALLS OF AMMONIUM URATE.

fine blue needles. The crystals of indigo are not rare in decomposing and fermenting urines, in which they result from the decomposition of the indoxyl-sulphate. They occur more especially in the urine of hepatic abscess and in cirrhosis of the liver.

Urinary concretions of considerable size are occasionally to be seen in urine, with the naked eye. They consist, for the most part, of acid urates, or urates with uric acid. Their recognition is important in the diagnosis of renal colic. When composed of uric acid or acid urates, their color is usually red or brown. Phosphatic concretions

of larger size occur more rarely. They are light-colored. Other concretions are occasionally met with.

Foreign bodies occur in the urine from accidental causes, or from negligence in collecting the specimen. We may mention fungi, yeast-cells, micro-organisms, fat globules; fibers of silk, linen, and wool; feathers, wood, starch, etc. Bodies of this kind will be seen in almost every specimen examined. They will not cause any confusion, after a little practice, as they are so different from any of the characteristic urinary sediments that mistakes will rarely be made. Fragments of tumors, as sarcoma, carcinoma, etc., may occasionally be found, and their import is self-evident.

Organized Deposits. — Mucous corpuscles are seen as round, finely granular cells, somewhat larger than blood-corpuscles, and containing

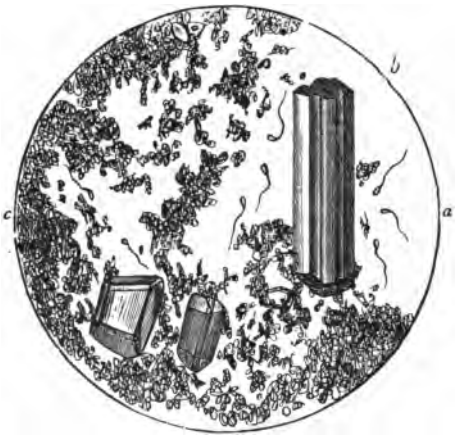


FIG. 22.—(*a*) SPERMATOZOA; (*b*) AMORPHOUS CALCIUM CARBONATE; (*c*) AMMONIUM MAGNESIUM PHOSPHATE.

from three to five nuclei. They can not be distinguished from the colorless blood-corpuscles. (See Fig. 28.)

Pus-corpuscles resemble the mucous corpuscles in their appearance.

If fresh, warm urine containing mucous or pus-corpuscles be examined under the microscope, the corpuscles will be seen to undergo the ameboid change of shape. When the urine becomes cold this movement ceases, and the corpuscles assume the globular form. Water causes these corpuscles to swell, the nuclei becoming more distinct, and the outline gradually disappearing. Acetic acid produces

a similar change, but more rapidly. Solutions of KOH, NaOH, and NH_4OH disintegrate these corpuscles, destroying the cells and granules; the nuclei are the last to disappear. In the ammoniacal urine of chronic cystitis they rapidly dissolve. This fact should be borne in mind in this disease.

It is a matter of great importance in every case where pus is found, to look for and identify any epithelial cells that may be present, as this is the only way of determining the source of the pus.

The character of the pus cells, whether normal or abnormal in appearance, is of importance.

It is claimed by Heitzmann that by the character of the pus corpuscles the vigor of constitution of the individual may be determined and thus becomes an important aid in prognosis.

According to him coarsely granular highly refracting corpuscles, when present in large numbers indicate "an excellent, first-class constitution."

When the greater number of the corpuscles are less coarsely granular, and a nucleus is seen, it indicates a medium constitution, while a finely granular condition with one or more pale nuclei, indicates a poor constitution. These distinctions can only be satisfactorily seen with a one eighth or one tenth inch objective. In greatly debilitated states of the system, and in cases approaching a fatal termination the pus cells break down completely.

Pus in the urine always indicates a suppurative process somewhere along the urinary tract, except in women, when it may get into the urine from the uterus or vagina. The pus may be derived from any part of the urinary passage.

It is sometimes difficult to determine its source, but the following points will aid in making the diagnosis: In blennorrhea of the urethra, a purulent fluid may be pressed out of the urethra between the micturitions, or the first few drops of urine passed will be nearly all pus. In this disease, too, the pus is found in shreds or clots in the urine. In purulent cystitis the other symptoms of the disease will usually be present, as frequent micturition, strangury, etc., and perhaps the last few drops of urine will contain a larger quantity of pus than the rest. The urine is apt to be alkaline, and contains a sediment when passed. There is usually pain in the hypogastric region and scalding pain in passing urine. There is not so much pain, however, as in acute cystitis. In suppuration along the course of the ureter there are usually attacks of slight colicky pains in this region. In abscess, the flow of pus is generally intermittent, and there is generally a tumor to be felt over the region of one or both kidneys, usually but one. Suppuration confined to the parenchyma of the kidney is often accompanied by very slight local symptoms. If the pus comes from the pelvis of the kidney, there are lumbar pains and absence of bladder symptoms, and the urine is acid. The presence or absence of renal casts will assist in making a diagnosis. There are seen transitional epithelial cells. If from the vagina, there will be leucorrhœal symptoms, abundant pavement epithelial cells, with excess of mucus, and acid urine, and absence of bladder symptoms.

The ability to recognize and differentiate the epithelial cells from the different parts of the urinary tract is of the greatest diagnostic importance, for upon this ability rests the diagnosis of most diseases of the urinary organs.

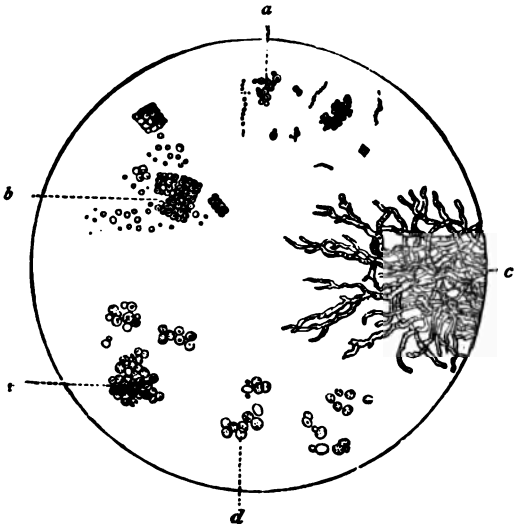


FIG. 23.—(*a*) MICROCOCCI IN SHORT CHAINS AND GROUPS; (*b*) SARCINÆ; (*c*) FUNGI FROM ACID FERMENTATION; (*d*) YEAST CELLS FROM DIABETIC URINE; (*e*) MYCELIUM OF A FUNGUS, OR MOULD.

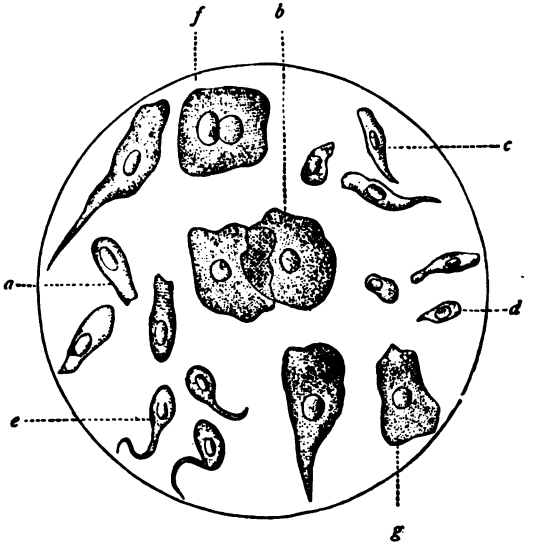


FIG. 24.—(*a*) EPITHELIAL CELLS FROM THE MALE URETHRA; (*b*) FROM THE VAGINA; (*c*) FROM THE PROSTATE; (*d*) COWPER'S GLANDS; (*e*) LITTRE'S GLANDS; (*f*) FEMALE URETHRA; (*g*) BLADDER.

FIG. 25. — CRENATED RED BLOOD-CORPUSCLES IN THE URINE. × 350.

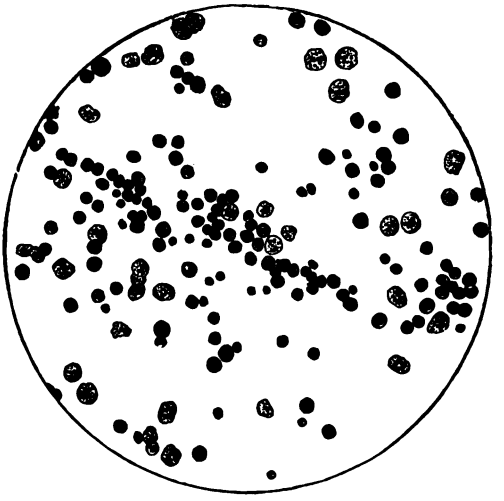


FIG. 26. — RED AND COLORLESS BLOOD-CORPUSCLES OF VARIOUS FORMS.

Epithelial cells of a variety of shapes are found in normal urine. Those from the convoluted portion of the tubules are polygonal in shape, but on remaining for some time in the urine they absorb water and become globular. They are about one third to one half larger than the pus-corpuscles, and may be distinguished from the latter by having but one large, distinct nucleus. The epithelial cells of the loop of Henlé and the larger collecting tubes are columnar in shape. Those from the ureter, pelvis, and male urethra are elongated and conical, containing one nucleus near the center. Large, flat, squamous, epithelial cells are obtained from the bladder, vagina, and female urethra. (Fig. 24.) In chronic cystitis, after the large, flat, irregular cells have been shed off, we have smaller, rounded cells. Rapidly proliferating cells have a large nucleus in proportion to the remainder of the cell. Old cells, slowly proliferated and desquamated, have a smaller nucleus in proportion to the rest of the cell. This is of importance in the diagnosis of new growths likely to be found in the bladder.

Blood-corpuscles in the urine appear as small, round, disc-shaped corpuscles of a light straw or red color, and when seen on the edge appear biconcave. They are prone to changes in form, color and size on standing for some hours. Blood corpuscles that have remained for some hours in a concentrated urine become crenated. (Fig. 25.) In some cases they become smaller in size, lose their color and appear as mere rings. Blood cells that come from the kidney are usually paler than those coming from the bladder or urethra, and the urine is darker in color than when it comes from lower down. Blood corpuscles undergo decomposition in alkaline urines, change their form, and finally become invisible. (Figs. 25 and 26.)

Renal casts. — Renal casts are also called *tube casts* and *cylinders*. They are produced by the formation of a coagulum of jelly-like consistency in the tubules of the kidney. This coagulum, more or less hardened and retaining the shape and size of the tube is pushed along in the tube by the urine into the larger tubes, the pelvis and the bladder. Whether they are composed of fibrin, or a special proteid formed by the injured epithelial cells of the tubules, or from their disintegration, has not been definitely determined. They may be clear and transparent or they may contain blood- or pus-corpuscles, epithelial cells, granular matter, crystals, or oil-drops, imbedded in the substance of which they are composed, from which they are named epithelial casts, blood-casts, granular casts, fatty casts, waxy casts, and hyaline casts.

Hyaline casts are perfectly clear, transparent cylinders, without markings, having nearly the same refractive index as the urine, and

consequently are not readily seen, especially in a strong light. (Fig. 28.) They are more readily seen with oblique illumination, or by adding a few drops of solution of eosin, Bismarck brown, methyl-green, or fuchsin, to the urine while the sediment is forming. They are characteristic of the very earliest and the recovering stages of nephritis, and are also found in congestion of the kidney, or in simple irritative catarrah of the tubules.

Blood-casts contain blood corpuscles imbedded in them, and indicate an acute inflammation of the kidney with escape of blood corpuscles from the circulation into the tubules. (See Fig. 32.) They are characteristic of the very acute stages of nephritis.

Epithelial casts are those in whose surfaces epithelial cells from the tubules are imbedded. (See Fig. 33.) They indicate a rapid shed-

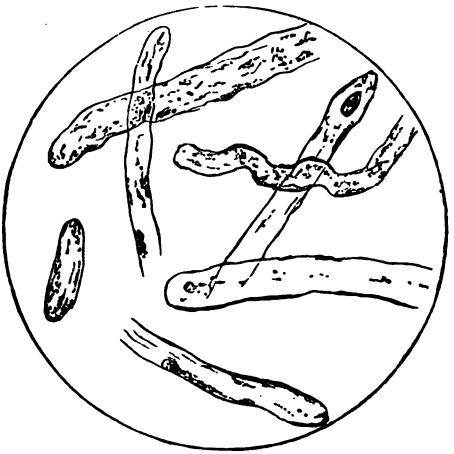


FIG. 31 —HYALINE CASTS.

FIG. 32.—BLOOD-CAST.

ding of the epithelial cells lining the tubules, and usually occur in the second stage of the inflammation — *i. e.*, when the inflammation has loosened the epithelial cells. They will usually be found ouly in acute nephritis.

Granular casts are those containing granules, either small or large. The granular matter may come from either the disintegration of the epithelial cells, blood-cells, or from the material of the cast itself. They are frequently spoken of as finely granular, moderately granular, and coarsely granular; the amount of granular matter giving an idea

of the amount of disintegration taking place in the kidney. The dense, coarsely granular varieties, represented by Fig. 34, *b*, are more especially found in chronic cases. The finely granular cast

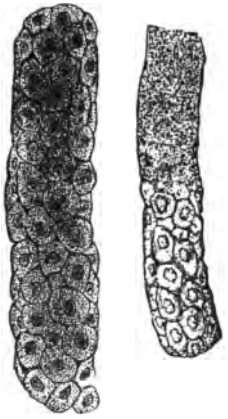


FIG 33 —EPITHELIAL CASTS.

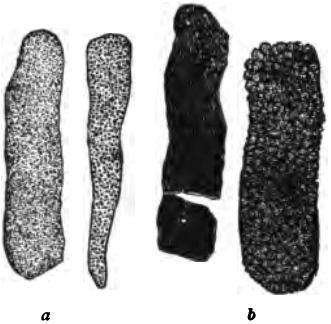


FIG. 34.—GRANULAR CASTS.

seen in Fig. 34, *a*, may be found in the subacute or chronic form of the disease.

Fatty casts, or **oil casts,** are such as reveal oil drops in the cast material. They occur in chronic nephritis attended by fatty degeneration. It is sometimes difficult to determine whether the granular degeneration seen in these casts is due to the degeneration of the cast itself, after having been formed, or whether it is the result of the disintegration of blood-cells or epithelial cells. These casts form in the tubules, and often remain there for a considerable time — a sufficient time, perhaps, to undergo granular and even fatty degeneration. It is certain that the coarsely granular and fatty casts are never found in the earlier stages of the disease.

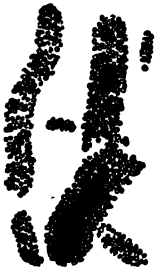


FIG. 35.

FIG. 36

Waxy casts are a variety somewhat resembling hyaline casts, but are more dense and more distorted, and frequently are cracked or torn along the edges, or they have lost the regularity of their outline. They

FIG. 37.—GOUTY NEPHRITIS. *a*, finely granular cast; *b*, uric acid; *c*, casts of acid urate of sodium. (BLUMENTHAL.)

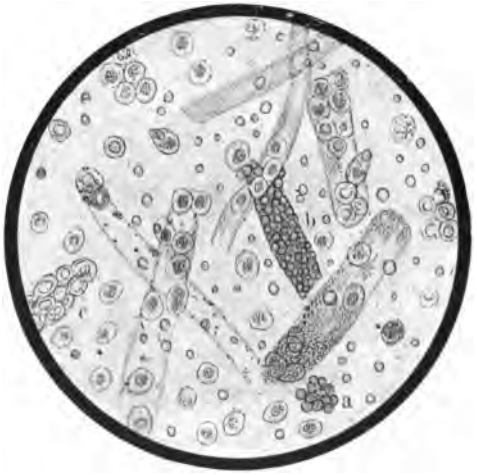


FIG. 38.—ACUTE DIFFUSE NEPHRITIS. *a*, red blood cells; *b*, blood cast; *c*, epithelial cast. (BLUMENTHAL.)

sometimes give a blue color with sulphuric acid and iodine, are more refractive than hyaline casts, and are insoluble in acetic acid, while hyaline casts are soluble.

FIG. 39.—CHRONIC PARENCHYMATOUS NEPHRITIS. *a*, waxy casts; *b*, epithelial casts. Renal cells. (BLUMENTHAL.)

FIG. 40.—FATTY PARENCHYMATOUS NEPHRITIS. Hyaline, granular, leucocyte, fatty and epithelial casts. Pigment masses, renal cells and fat drops. (BLUMENTHAL.)

Mucous casts or **cylindroids** are frequently spoken of. They are long, transparent, fibrillar bodies, twisted and branching, and lacking

FIG. 41.—FATTY DEGENERATION OF CASTS AND EPITHELIAL CELLS.

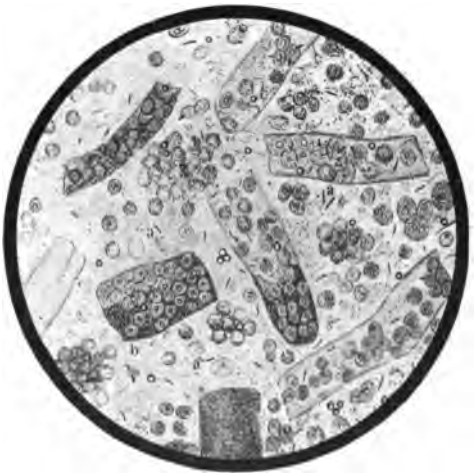


FIG. 42.—SEPTIC NEPHRITIS. *a*, bacilli; *b*, epithelial casts, renal cells, leucocytes and hematoidin-crystals. (BLUMENTHAL)

in the terminal features of casts. They should not be regarded as casts, although we may meet with mucous plugs from the follicles in

the prostatic urethra which closely resemble casts. The character of the epithelial cells, with which they are associated, will usually serve to distinguish them. The absence of albumin will also assist. True renal casts without albumin in solution are rare. Hyaline casts without albumin are not rare, but they are frequently mucous cylinders instead of true tube-casts.

Casts can usually be readily distinguished from other bodies met with in the urine by the peculiarly rounded end, formed by the pushing of the cast material through the tubule by pressure from behind, while still in a plastic condition. This rounded extremity is one of the most characteristic features in casts, and when in doubt as to the identity of an object, this will often serve as a guide.

False or Pseudo-casts. — Casts like formations of **urates** will occasionally be met with, and always resemble granular casts. (See Figs. 33 and 37.) Masses of micrococci closely resembling casts will also occasionally be seen, but these can usually be distinguished by their appearance, or by their resistance to reagents, as caustic potash, nitric acid, etc. **Leucocyte casts** (Fig. 36) are met with in suppurating conditions of the tubules of the kidney. Similar looking bodies are sometimes seen in gonorrhea, prostatitis, and leucorrhea.

Granular Detritus. — Under this name we will desiginate the ill-defined granular or disintegrating masses of material frequently met with in cases of nephritis. These irregular or amorphous masses are probably disintegrated cells, or masses of free granules of this origin. The amount of this material in any specimen of nephritic urine should be noted, as an aid in arriving at a clear idea of the amount of destructive change going on in the kidney. This point is an important one in prognosis, as by it we are able to determine that organic destruction of the kidney is rapidly progressing and the prognosis unfavorable; or, that there is little or no organic destruction and the prognosis better. Fibres of connective tissue may often be found in the urine of nephritis, which will indicate destruction of the deeper structures of the urinary tract. They occur in contracted kidney, suppuration, ulceration and new growths.

Figs. 37 to 41 will illustrate the microscopic pictures to be expected in some of the more common renal diseases. The student is advised to study these in connection with the descriptions of the urinary findings on pages 83 to 86.

SYSTEMATIC SCHEME FOR THE CHEMICAL AND MICROSCOPICAL EXAMINATION OF SEDIMENTS.

CHEMICAL EXAMINATION.

Draw off a portion of the sediment with a pipette or glass tube, and transfer to a watch-glass or small test-tube.

- **White Deposit.**
  - Dissolves on heating urine, ... *Ammonium urate.*
  - Insoluble on heating.
    - Sol. in NH_4OH, ... *Cystin.*
    - Insol. in NH_4OH,
      - Soluble in acetic acid, *Earthy Phosphates.*
      - Insoluble in acetic acid, *Calcium oxalate* or *oxalurate.*
    - Gelatinizes in NH_4OH, *Pus* (see above).
- **Colored Deposit.**
  - Visibly crystalline (red), ... *Uric acid.*
  - Amorphous,
    - Pale, easily soluble by heat, ... *Urates.*
    - Deep-colored, slowly soluble by heat, *Acid urates with uroerythrin.*
    - Red, insoluble by heat, alkalies, or acids, . . . *Blood.*

MICROSCOPICAL EXAMINATION.

With a clean pipette draw off a small portion of the sediment, transfer to a clean glass slide, and examine with a ½ in. and then ⅙ in. objective.

- **Deposit is Amorphous.**
  - Small granules with spicules or larger granules; vanishes on adding KOH or NaOH, ... light = *Sodium urate.* dark = *Ammonium urate.*
  - Permanent on adding KOH or NaOH, . . *Calcium carbonate* (rare).
  - Globules, strongly refracting light, ... *Fat.*
- **Deposit is Crystalline.**
  - Urine, Acid.
    - Yellow, cross or whetstone shaped, or in groups, *Uric acid.*
    - Regular octahedra, envelope-shaped, . . . *Calcium oxalate.*
    - Hexagonal plates, soluble in NH_4OH (white), . . . *Cystin.*
    - Bundles of needles crossing each other, ... *Tyrosin.*
  - Alkaline Urine.
    - Large prisms, soluble in acetic acid (coffin-lid shaped), *Ammonium magnesium phosphate*
    - Brown, double spheres, spiculated, *Urate of ammonium.*
    - Club-shaped crystals, single or in groups, *Calcium phosphate.*
    - Double spheres, radiated structure, soluble in acetic acid, with effervescence, . . *Calcium carbonate* (rare).
    - Double spheres, insoluble in acetic acid, *Calcium oxalurate* (rare).
    - Double spheres, yellow or red, striated, . . *Uric acid.*
- **Cellular Elements.**
  - Red or yellow discs, biconcave; sometimes irregular in outline, *Blood-cells.*
  - Granulated corpuscles. With diluted acetic acid, show 3 to 5 nuclei, Albumin present, ... *Pus.* Albumin absent, *Mucous corpuscles.*
  - Round, conical, or flat cells with one nucleus, *Epithelium from urinary tract.*
  - Tadpole-shape, with long tail (small), ... *Spermatozoa.*
  - Cylinders, parallel margins, clear, granular, or containing epithelial cells or blood-cells, ... *Casts of uriniferous tubules.*
  - Fungi, yeast, hairs, threads, etc., etc., ... *Extraneous matters.*

URINARY CALCULI.

Urinary calculi, or concretions, are hard masses of urinary sediments formed in the kidney, ureters, bladder, or sinuses of the prostate gland. They are **simple**, composed of one kind of material, or **compound** or **mixed**, composed of two or more kinds of material, deposited in concentric layers. In the examination of a calculus it should be sawed through so as to expose these layers, and small portions of each layer examined separately. An examination of a calculus is necessary to determine the condition which led to its formation, and to suggest proper treatment to prevent the formation of others. *Uric acid or acid urates* compose about sixty per cent. of all urinary calculi. They are generally reddish and smooth, but sometimes tuberculated. About forty per cent. of the remainder of the stones are mixed uric acid and earthy phosphates, containing more of the latter. When the calculus starts as a uric acid concretion, and the urine changes from acid to alkaline, the phosphates are deposited. This is apt to occur sooner or later. The cross-section of such a calculus shows very plainly the different layers.

Calcium oxalate calculi, or **mulberry calculi,** comprise about three per cent. of all cases operated upon. They are gray or dark brown, very hard, and generally tuberculated, when they are often called "mulberry calculi." When smooth they are often called "hempseed calculi."

The phosphatic calculi are rare, as are those composed of calcium carbonate, cystin, xanthin, fibrin, blood, indigo, and urostealith.

These last, composed of calcium and magnesium soaps, fat, albumin, etc., are very rare.

SCHEME FOR THE QUALITATIVE EXAMINATION OF CALCULI.

Heat a portion of the powdered stone on a platinum-foil, or charcoal, with blowpipe.

A. *It chars and burns with a flame.* Probably xanthin, cystin, urostealith, or fibrin.

1. The flame burns briefly, emitting odor of SO_2. The powder dissolves in ammonia, and on diluting deposits six-sided plates = **Cystin.**

2. It does not give the murexid test. The powder is soluble in HNO_3, without effervescence, and the dried residue becomes orange with alkalies and red on warming = **Xanthin.**

3. The flame is yellow, prolonged, and gives the odor of burning shellac. The powder is soluble in alcohol = **Urostealith.**

4. The flame is yellow, prolonged, and gives the odor of burning feathers. Soluble in hot NaOH solution, and is precipitated again by acidifying with acetic acid = **Fibrin.**

B. *It chars, but does not burn with flame.*

1. The powder gives the murexid test. *a.* It gives off NH_3 when

warmed with NaOH solution = **Urate of Ammonium.** *b*. It gives no NH_3 with NaOH = **Uric Acid**.

C. *The powder does not strongly char or burn.* Treat with diluted HCl.

1. It dissolves with effervescence = **Calcium Carbonate.**

2. It dissolves without effervescence, and the solution gives a white precipitate with NH_4OH = **Phosphates** or **Calcium Oxalate.**

Treat the powder with acetic acid.

Phosphates dissolve without effervescence.

Mixed phosphates fuse in heating on foil.

Calcium phosphate does not fuse.

Triple phosphate gives off NH_3 when warmed with a little NaOH solution.

Calcium oxalate is insoluble in acetic acid. After ignition it gives an alkaline powder, which effervesces with acetic acid or diluted HCl.

THE URINE OF THE TWENTY-FOUR HOURS—NORMAL AND PATHOLOGICAL.

PHYSICAL CHARACTER.	NORMAL.	ALTERATIONS IN ABNORMAL CONDITIONS.
Color.	Pale straw to reddish yellow. The average color is amber.	Colorless: neuroses, chronic nephritis, diabetes. High-colored: acute fevers, icterus. Blood-red: blood or foreign color. Dark brown: hematuria, poisoning by carbolic acid, potass. chlorate, or iodoform. Smoky brown: presence of decomposed blood, acute nephritis. Yellow or green: presence of bile. White: chyle or pus.
Transparency.	Clear, with only a slight flocculent cloud of mucus.	Urine turbid when passed, is abnormal. Whitish sediment may be pus, phosphates, or ammonium urate.
Consistence.	When normal, urine is mobile, like water.	When viscid, it indicates albumin, bile, mucus, or pus.
Odor.	Peculiar to itself.	Urine putrid when passed, indicates cystitis.
Reaction.	Slightly acid; becomes more acid on standing, then becomes alkaline.	Urine strongly acid in fevers and inflammations of the liver, heart, and lungs; in acid dyspepsia. Urine is alkaline in cystitis, and occasionally in debility, chlorosis, certain organic nervous diseases, typhus, etc.

THE URINE OF THE TWENTY-FOUR HOURS—NORMAL AND PATHOLOGICAL.—*Continued.*

CONSTITUENTS.	AMOUNT IN GRAINS.	ALTERATIONS IN PATHOLOGICAL CONDITIONS.
Sulphuric acid.	23 to 38	Having more or less the same source as urea, it will increase or diminish therewith. Occurs as sulphuric esters, and preformed.
Phosphoric acid.	46 to 54	**Increased** in fevers, in nerve-exhaustion, disease of spinal cord, and in tubercle of the lung. In phosphatic diabetes the alkaline phosphates are greatly increased. **Diminished** in many mental diseases, especially mania, and in chlorosis.
Oxalic acid.	0.3	**Increased** in catarrhal jaundice, and in oxalic acid diathesis, mental depression, and certain forms of dyspepsia.
Phosphate of lime.	4 to 5	**Increased** in osteomalacia, rickets, scrofula, carcinoma, long-continued suppuration, organic disease of the spinal cord. **Diminished** in fevers.
Phosphate of magnesium.	7 to 11	
Chloride of sodium.	150 to 250 (Cl = 90 to 150, Na = 60 to 90)	**Increased** in fevers at the outset, and with the reabsorption of dropsical fluids. **Diminished** during apyrexia, dropsies, cholera, typhus, inflammations generally, and especially in the forming stage of pneumonia.
Free acid (calculated as oxalic acid).	30 to 60	**Increased** during the acme of acute febrile affections (on account, probably, of the diminished proportion of water present). **Diminished** in most diseases affecting the nutrition and leading to a deficiency thereof.
Total inorganic salts.	200 to 380	
Potassium.	38 to 48	
Sodium.	140 to 180	
Calcium.	4 to 5	
Magnesium.	2 to 3	

ORGANIC CONSTITUENTS.

Urea.	450 to 500 or 30 to 34 gm.	**Increased** after much meat, in fevers, diabetes mellitus, copious drinking of water or alcohol, congestion of liver. **Diminished** in abstinence from meat, rest, hepatic abscess, nephritis, chronic wasting diseases.

THE URINE FOR THE TWENTY-FOUR HOURS—NORMAL AND PATHOLOGICAL.—*Continued.*

Organic Constituents.	Amount in Grains.	Alterations in Pathological Conditions.
Uric acid.	4 to 15 (ratio to urea, 1 : 40)	**Increased** in leucocythemia, pernicious anemia, gout, rheumatism, deficient oxidation; organic diseases of heart, lungs, liver, or skin; after acute fevers and excessive meat diet. **Diminished** in vegetable diet, gout, before attack, chronic renal disease, outdoor exercise.
Hippuric acid.	5 to 15	**Increased** with vegetable diet, after taking benzoates. **Decreased** in animal diet.
Creatinin.	8 to 15	**Increased** in meat diet, and increased nitrogenous metabolism. **Decreased** in vegetable diet, and milk diet.
Xanthin Bases.	0.5 to 2	**Increased** in splenic disease, meat diet, deficient oxidation. **Decreased** in vegetable diet.
Carbolic acid, Cresol, etc.	0.015	**Increased** in certain diseases of the intestines, causing constipation (ileus, etc.), but has been observed to be increased also in certain cases of diarrhea.
Indoxyl.	0.07 to 0.05	**Increased** with diseases attended by constipation and intestinal fermentation, and occasionally, also, in cases of diarrhea. After cholera, cancer of the liver and stomach, purulent peritonitis. Valuable diagnostic sign in typhoid fever and cancer of the liver.
Acetone, Diacetic acid, Hydroxybutyric acid.	Traces. " "	**Increased** in diabetes mellitus; conditions of increased proteid metabolism, with deficient oxidation.
Albumin.	None.	Nephritis, pregnancy, poisoning by certain substances, cold baths, violent exercise, rheumatism, infectious fevers, etc.
Albumose.	None.	Presence not clearly diagnostic.
Peptone.	None.	" " " "
Dextrose.	None or trace.	Glycosuria and diabetes mellitus.
Lactose.	None.	During lactation, after weaning.
Bile.	None.	Obstruction in bile-duct, structural hepatic diseases, malaria, pernicious anemia, yellow atrophy of liver, typhoid fever, and AsH_3 poisoning.
Blood.	None.	Hemorrhages, giving hematuria; hemoglobin in malaria, acute nephritis (sometimes).
Pus.	None.	Suppuration.
Mucus.	Present.	**Increased** in any irritation along the urinary tract, by uric acid, calcium oxalate, etc., catarrh of bladder, urethra, vagina. Usually increased in acute fevers.

The foregoing table gives the most prominent variations in physical and chemical characters of the urine, with brief notes of their significance.

THE GASTRIC CONTENTS.

The clinical examination of the stomach and its contents, for diagnostic purposes, generally includes an estimation of the rapidity of absorption, the motility, the reaction, and acidity of the gastric juice; the determination of the kind and amount of acids present; the determination of the digestive activity, and a microscopical examination of the stomach contents.

The stomach contents for examination are drawn off with a stomach tube after the administration of a test meal of approximately known composition.

Ewald's Test Breakfast. — This is usually given in the morning and consists of an ordinary dry roll and a definite quantity — say, 300 c.c., or about ⅔ of a pint — of fluid, either simple warm water or weak tea without milk or sugar. The roll should be of a tolerably uniform weight of about 35 grms., or 540 grains. Such rolls contain about 7 per cent. of proteids, 0.5 per cent. of fat, 0.4 per cent. of sugar, 52.5 per cent. of non-nitrogenous matter, and about 1 per cent. of ash.

Boaz's Test Breakfast consists of a pint of oatmeal gruel made by boiling an ounce of oatmeal with water, and salted to taste. This meal contains no lactic acid and should be used when this acid is to be tested for.

A similar gruel made with barley has been recommended by some.

About one hour after taking the above test-breakfast the stomach tube is inserted, and that which remains in the stomach is drawn off. The amount obtained varies considerably even in health, but from 20 to 50 c.c. can usually be obtained. A larger amount indicates dilatation of the stomach, or deficient motility.

Leube and **Riegel** advocate a much more complicated meal or **test-dinner.** It consists of a plate of soup (about 350 c.c.), 60 grms. of minced or scraped meat, and one or two slices of bread, say 40 to 50 grms. The contents are removed about four hours after such a test dinner. It is evident that the results will vary somewhat when these different meals are used.

Method of Drawing Out the Contents of the Stomach for Chemical and Microscopical Examinations. — The contents of the stomach are usually drawn off about one hour after the person has taken one of the above test-breakfasts. This is accomplished by the stomach-tube, Einhorn's gastric bucket, or by causing the patient to vomit. The

first of these methods is usually to be preferred. Place the patient in a sitting position with his clothing protected by macintosh or other waterproof material. Dip the rounded end of the elastic stomach-tube in warm water; hold it about six inches from its extremity, and, as the patient opens his mouth, pass it to the back of his throat, and ask him to swallow. As he does so, pass the tube gently but rapidly into the esophagus, and onward to the stomach. If the gastric contents do not run out as soon as the outer end of the tube is lowered, cause the patient to lean forward, compress the abdomen and make an effort to retch, when it will cause the contents to run out.

Examination of Stomach Contents. — Observe the quantity, the consistency, color, amount of mucus, and odor. Note any fragments of former meals, if such be present, or fragments of gastric membrane or blood. Fragments of membrane should invariably be examined under the microscope, for evidences of organic disease. Filter the stomach-fluid, and preserve the solid portion for further examination. It is advisable in some cases to first strain the contents through clean dry muslin, and then pass the filtrate through paper. The chemical tests should be carried out on the clear filtrate as soon as possible. The following order of tests will be the most convenient:

1. Macroscopic appearance.
2. Amount.
3. Odor (sour, butyric, putrid).
4. Undigested food and its character.
5. Mucus, blood or pus.
6. Reaction.
7. Qualitative test for free HCl.
8. Estimation of total acidity.
9. Estimation of free HCl, combined HCl, and acid salts.
10. Presence of lactic acid.
11. Total organic acids.
12. Presence of pepsin and its activity.
13. Presence of rennin or milk-curdling ferment.
14. Presence of starch, dextrin, and maltose.
15. Presence of proteids and their nature.
16. Microscopical examination of the sediment.

Reaction. — The normal reaction of gastric juice is decidedly acid, and the acidity is due to HCl, acid salts (H_2NaPO_4), organic acids, and occasionally carbonic acid. Lactic acid is usually present after an ordinary meal, but after the oatmeal test-breakfast it is usually absent, except in carcinoma. There are two sources of lactic acid — one from fermentation of the contents, especially liable to occur when the motor function of the stomach is deficient and the food is retained longer than usual; the other, sarcolactic acid, introduced with meats and other articles of diet. Butyric and acetic acids are occasionally found in the stomach contents, due to these fermentations. The reaction of the fluid is determined with litmus paper. Unsized paper

stained with other coloring matters may be used (p. 47). The following table gives the indicators most frequently used in the examination of gastric fluid, with indications:

Name of Color.	Solvent.	Color with Acids.	Color with Alkalies.	Reacts with:	Degree of Sensitiveness.
Phenolphthalein, ...	Alcohol, 1 per cent.	Colorless.	Pink or Red.	All acids.	Very sensitive.
Congo-red, ...	Water, 1 per cent.	Blue.	Wine Red.	Free acids only.	HCl=0.1 in 1000. Lactic=0.2 in 1000.
Tropeolin, ...	Water.	Deep Red.	Yellow.	All acids.	HCl=0.3 in 1000.
Litmus, ...	Water.	Red.	Blue.	All acids.	Very sensitive.
Dimethyl-amido-azobenzene, .	Alcohol, 0.5 per cent.	Cherry Red.	Yellow.	Free mineral acids.	HCl=0.02 in 1000.
Alizarin-sulphonate of sodium,	Water, 1 per cent.	Yellow.	Violet.	Free acids and acid salts.	. .
Boas' Resorcin Solution, . . . [Resorcin, 5 gm.; cane-sugar, 3 gm.; alcohol (95 per cent.), 100 c.c.]	Alcohol.	Pink.	Colorless.	Free mineral acids.	HCl=0.05 in 1000.
Gunzburg's Solution, ... [Phloroglucin, 2 gm.; vanillin, 1 gm.; alcohol (95 per cent.), 100 c.c.]	Alcohol.	Pink.	Light Brown.	Free mineral acids.	HCl=0.05 in 1000.

Litmus paper is reddened by all acids and acid salts. Congo-paper turns blue in presence of all free acids, and dimethyl-amido-azobenzol is reddened by free mineral acids only. By testing the filtered gastric contents successively with paper colored with these three colors, we may determine whether it be acid, with litmus; whether the acidity be from a free acid, with congo-red; and whether from free HCl, with azobenzene paper. When the congo-paper is blued by the solution, showing free acid, and the paper is warmed gently over a flame, the blue color is discharged when it is due to volatile organic acids alone, but remains blue if it has been produced by hydrochloric acid. We may confirm the presence of HCl, if it is thought necessary, by the solution of Boaz or that of Gunzburg, as described below (p. 138).

Detection of Organic Acids. — The presence of a considerable amount of organic acids in the stomach contents after the usual test-meal is to be regarded as pathological. The acids present may consist of lactic, butyric, or acetic acids. *Butyric acid* can usually be

detected by an odor like that of rancid butter. *Acetic Acid* will be detected by the odor of vinegar, especially on warming the fluid. The detection of the organic acids is more certain when they are separated from the fluid by shaking them out with ether, allowing the ether to evaporate and applying the tests to the residue. As *lactic acid* is likely to occur in larger amounts and more frequently than the others, several tests have been proposed for its detection. Any pronounced quantity of organic acids is generally regarded as an evidence of pyloric stenosis, deficient HCl secretion, deficient motor power, or dilatation of the stomach — in other words, of an unusual delay of food in the stomach, with resulting fermentations. When we desire to make a careful clinical test for lactic acid, it is best to give a test-breakfast of oatmeal or barley-gruel, in preference to Ewald's test-breakfast, as the former contains no lactates, while the latter may.

There are two lactic acids met with in the stomach — sarcolactic acid and fermentation lactic acid. Sarcolactic acid is formed in the stomach from meat taken as food; the other lactic acid may be formed by fermentation, or it may be introduced with such foods as milk, sour cabbage, bread, etc. A very simple and rapid test for clinical purposes is the following:

Uffelmann's Test for Lactic Acid. — A few drops of a dilute neutral solution of ferric chloride are mixed with two drops of pure carbolic acid, and water added until the solution assumes a beautiful amethyst-blue color. A few drops of a 1 : 2000 solution of lactic acid instantly changes the color to yellow. The delicacy of the reaction is very great. Lactates, as well as free lactic acid, unfortunately, produce this yellow color. It is said that alcohol, sugar, and certain salts can produce the same color. The test becomes certain if we first extract the organic acid from the gastric contents with ether, and apply the test to an aqueous solution of the residue left on evaporating off the ether. Acetic and butyric acids can usually be detected by the odor. The odor of acetic acid is usually unmistakable. It may also be detected by the use of ferric chloride, with which it gives a red color. Butyric acid changes Uffelmann's reagent to a grayish, opalescent color, and this only occurs when it is present in over 5 parts per 1000. Alcohol is found only in rare cases of yeast fermentation.

Another delicate test for lactic acid is the following: Two or three drops of Fe_2Cl_6 solution are diluted with distilled water until the yellow color is almost imperceptible. 1 c.c. of the suspected solution is now added, when the yellow tint is restored, if lactic acid be present. Butyric, acetic or hydrochloric acids do not give this appearance unless in strong solution.

Total Acidity. — The acidity of the gastric contents during digestion is made up of free HCl, free organic acids, acid albumins, consisting of a loose combination of HCl and organic acids with the proteids of the food, and acid salts, chiefly acid sodium phosphate, NaH_2PO_4.

Estimation of Total Acidity. — To 10 c.c. of the filtered fluid, accurately measured into a beaker, three drops of a 1 per cent. solution of

phenolphthalein is added, and enough $N/10$ NaOH solution, accurately measured from a burette, to produce a permanent pink color. After the addition of a few cubic centimeters of the decinormal soda solution, a light rose color appears, which should not be mistaken for the end reaction. The final change of color is produced by a single drop of the alkali, and hence the addition should be made drop by drop near the end. Near the completion of the test, each drop will produce a pink-red cloud as it falls into the liquid, which will disappear on gently mixing the contents of the beaker by a rotary motion.

Estimation of Free Hydrochloric Acid. — Many methods have been devised for the estimation of the free hydrochloric acid, some of which are complicated and troublesome, while others are comparatively simple and require little skill. **Simplicity of manipulation, reasonable accuracy of results, and a small consumption of time are prerequisites of a good clinical method.**

We shall omit complicated and tedious methods.

The Phlorogeucin-vanallin method of Gunzberg, and the Resorcin method of Boaz are conducted in exactly the same way and give the same indications. They are generally considered reliable methods, and are easy to manage. The composition of these reagents is given in the table of indicators above.

The Process: To 10 c.c. of the filtrate from the gastric contents add $N/10$ NaOH until a drop of the solution, evaporated on a white surface with the resorcin solution, fails to give a pink color. A piece of white "milk glass" answers very well for this purpose. A few drops of the indicator are spread over the glass and dried at a gentle heat. In performing the titration, a drop of the solution is removed on a glass rod and drawn across the plate, and the plate warmed over a naked flame. Blowing upon the streak as it evaporates hastens the appearance of the pink color.

The number of c.c. of decinormal NaOH solution used, multiplied by 0.00365, the weight of HCl neutralized by 1 c.c., gives the weight of free HCl in 10 c.c. This, multiplied by 10, gives the weight in 100 c.c. Or, the number of c.c. of NaOH solution multiplied by 10 gives the number of c.c. required by 100 c.c. of the filtrate.

Töpfer's Method of Estimating the Free and Combined HCl. — This method of estimating free and combined HCl is simple and easy enough to be adopted as a clinical method. It requires three separate titrations, practiced upon three portions of 10 c.c. each, using three different indicators. Three portions of 10 c.c. are accurately measured into three small beakers. In No. 1 phenolphthalein is used as an indicator, and the end reaction gives the total acidity, as described

above. In No. 2 dimethyl-amido-azobenzene, which reacts only with free HCl, is used as the indicator. In No. 3 alizarin-sulphonate of sodium is used as the indicator, which reacts with all the elements of acidity except acid albuminates.

The details of the method are as follows:

The total acidity is estimated in the *first portion* as described above.

To the *second portion* of 10 c.c. three or four drops of a 0.5 per cent. alcoholic solution of dimethyl-amido-azobenzene are added, and decinormal NaOH solution is run in from a burette until the color changes from red to a clear lemon-yellow. This color is very sensitive to mineral acids, and is not affected by combined HCl, acid salts, or by organic acids, unless the proportion of lactic acid reaches 0.2 per cent. or above.

When the estimation of organic acids to be described below requires the addition of three or more c.c. of decinormal NaOH solution for 10 c.c. of the fluid, it will be best to confirm the above titration with the resorcin solution of Boaz, or the phloroglucin solution of Gunzberg.

When the amount of gastric contents to be examined is small, the free HCl and the total acidity may be estimated in the same portion, using the dimethyl-azo-benzene first, and when the free HCl has been titrated, a few drops of the phenolphthalein are added and the titration continued. The presence of the first indicator does not interfere with the second.

The *third portion* of 10 c.c. is then colored with three drops of a 1 per cent. aqueous solution of alizarin (alizarin-monosulphonate of sodium) and titrated with *N*/10 NaOH until a clear reddish-violet color is reached. This is reached when the free HCl, organic acids, and acid salts have been neutralized. The difference between the number of c.c. used in this titration and that used in estimation of the total acidity with phenolphthalein, gives the number of c.c. of *N*/10 NaOH used in neutralizing the combined HCl.

Owing to the difficulty of an inexperienced eye in detecting the correct shade of violet in this titration, Töpfer recommends that a one per cent. solution of Na_2CO_3 be colored with the sodium alizarin-sulphonate solution, and this used for comparison, the color of this solution being imitated in the titration. The neutral tint is more nearly obtained in a one per cent. solution of sodium phosphate.

The above methods, after a little experience, give reliable results for clinical purposes, and require little skill in the manipulation.

Calculation and Statement of the Results.—The results may be stated in per cent., or parts in 100, or, as is more commonly done,

and quite as useful in practice, the results are stated in the number of c.c. of the $N/10$ NaOH solution required for 100 c.c. of the gastric contents.

Example.—In the titration of the free HCl with azo-benzene, 3 c.c., $N/10$ NaOH were used. This would require 30 c.c. for 100 c.c. or, as 1 c.c. of $N/10$ alkali neutralizes .00365 grms. of HCl, 30 c.c. will neutralize 0.1095 grms. or one tenth per cent.

In titrating this same specimen for the total acidity, with phenolphthalein as indicator, 10 c.c. gastric contents required 8 c.c. $N/10$ NaOH. 100 c.c. would require 80 c.c.

$.00365 \times 80 = .2920$ grms. acidity expressed in terms of hydrochloric acid.

With alizarin as an indicator 10 c.c. required 5.5 c.c. alkali. The combined HCl, then, was the difference between the NaOH solution used with alizarin and that used with phenolphthalein, or the total acidity. Or, 80 c.c. — 55 c.c. = 25 c.c. That is, 25 c.c. decinormal alkali would be required to neutralize the combined HCl in 100 c.c.

$.00365 \times 25 = .09125$ grms. The results in tabular form are:

Total acidity	80, or	.292 per cent.
Free HCl	30, or	.109 " "
Combined HCl	25, or	.0912 " "

Leo's Process of Estimating the Free and Combined Acids and Acid Salts.—This process depends upon the fact that when pure precipitated chalk, $CaCO_3$, is added to the fluid, the free acids and acids combined with albumin are neutralized by the $CaCO_3$, but not the acid salts. The acidity remaining after this treatment is due to acid salts only. By removing the organic acids with ether and then applying the method, the free and combined HCl may be estimated.

Ten c.c. of the filtered gastric contents are shaken in a separating funnel with 50 c.c. of ether, which dissolves the organic acids; the liquid is then separated from the ether, returned to the separating funnel, and again shaken with 25 c.c. of ether and again separated. The fluid is then treated with 5 c.c. of a twenty per cent. solution of $CaCl_2$, and titrated with $N/10$ NaOH, using phenolphthalein as the indicator. The result gives the acidity due to acid salts, plus the free and combined HCl. Another portion of 15 c.c. of the fluid is treated with about 1 grm. of $CaCO_3$, well mixed, and filtered through a dry filter into a dry flask. To 10 c.c. of the filtrate add 5 c.c. of the $CaCl_2$ solution and titrate with $N/10$ NaOH with phenolphthalein. The result gives the acidity due to acid salts, which, deducted from the result obtained in the first titration, gives the free and combined hydrochloric acid. The free HCl may be estimated by titration with azobenzene as an indicator.

Clinical Variations. — The hydrochloric acid is greatly diminished or entirely absent in the acute stage of all fevers; in chronic gastric catarrh with atrophy of the gastric glands and amyloid degeneration of the membrane; in all cachectic states, chlorosis, certain nervous troubles, many forms of poisoning, Addison's disease, cancer of the stomach, if it involves a considerable area or is attended with catarrh of the mucous membrane, which is usually the case. It is absent, as a rule, in cancer of the stomach, and this fact is a valuable aid in the early diagnosis of this disease. An excessive secretion of HCl is known as *hyperchylia*, or *hyperchlorhydria*, and is generally regarded as a neurosis.

Estimation of Organic Acids. — There are a number of such methods, but the most satisfactory is that of Hehner and Seemann, sometimes called Braun's method: Exactly neutralize 10 c.c. of the fluid with $N/10$ NaOH, and evaporate to dryness on a water-bath, in a platinum or porcelain basin. That portion used to determine the total acidity is usually employed for this process. When dry, the basin is heated over the lamp as long as the residue burns with a flame. The residue, after cooling, is extracted with boiling distilled water, filtered, and the filtrate titrated with decinormal HCl. The titration is best done by adding a measured excess of the $N/10$ HCl, the solution boiled to expel the carbon dioxide, and the excess of acid determined with $N/10$ NaOH, using phenolphthalein as the indicator. The difference between the number of c.c. of acid and alkali used will give the acidity due to organic acids present in the 10 c.c. of liquid taken. The organic salts of sodium formed during the neutralizing of the liquid are changed, by the ignition of the dry residue, into sodium carbonate. The amount of sodium carbonate present in the residue, which is estimated in this titration, is the measure of the amount of the organic salts formed. The above method leaves little to be desired as to its accuracy and simplicity.

Acetone. — Acetone is quite constantly found in the stomach contents in organic diseases of that organ. It is not found in functional diseases or in the the gastric neuroses.

Detection. — Acetone may be detected by distilling a portion of the contents, after the addition of 0.1 per cent. phosphoric acid. Lieben's or Reynold's test may then be applied to the distillate. Denige's test may be applied directly to the filtered gastric contents (see under Urine, page 94).

The Volatile Fatty Acids. — When it is desired to know the quantity of these acids present, they may be estimated as follows: Ten c.c. of the filtered gastric contents are evaporated on a water-bath to a syrup, made up again to about the original volume with pure water, and the acidity determined with $N/10$ NaOH, using phenolphthalein as the indicator. The difference between the acidity here determined

and the total acidity gives the volatile fatty acids. When lactic acid is known to be present, the difference between the total organic acidity and the acidity due to volatile fatty acids, may be taken to approximately represent the acidity due to lactic acid.

The Ferments — Pepsin. — The test for pepsin is accomplished by the addition of coagulated egg-albumin to the filtered gastric contents, and keeping this mixture at a temperature of about 40° C. (104° F.) for a definite time, and noting whether the albumin is corroded. If the gastric contents have been found by the above tests to be deficient in HCl, enough should be added to bring the quantity up to about two parts per thousand.

Pepsinogen. — In the absence of free HCl in the gastric contents, pepsin may be absent, but pepsinogen may be present, which only needs the addition of HCl to develop the pepsin. It is best to make two tests in such cases — one of the original fluid, and another after adding two drops of diluted HCl (U. S. P.). Coagulated albumin discs, made by cutting the white of boiled eggs in thin flakes of uniform thickness and punching them out by means of a cork-borer or glass tube, and preserving in glycerin, are used for this purpose.

Rennin or Chymosin. — The presence of rennin is best shown by carefully neutralizing 5 c.c. of the filtered gastric contents, and mixing 1 c.c. of this solution with 10 c.c. of carefully neutralized milk. If rennin be present, the milk is coagulated in fifteen minutes. In normal conditions this coagulation will take place when the above mixture of milk and gastric contents are diluted to 30 c.c.

Chymosinogen. — To 5 c.c. of the neutralized gastric contents add 10 c.c. of milk, and 2 c.c. of a 1 per cent. solution of $CaCl_2$ and warm to about 40° C. The presence of chymosinogen will be shown by coagulation in a few minutes.

The presence of chymosinogen is a fairly reliable proof of the absence of extensive organic disease of the stomach.

Digestion of Starch. — In normal digestion there is no free acidity, after the usual test-meal, for twenty to forty minutes. The salivary diastase continues its activity during this time, converting the starch into dextrin and maltose. At the end of one hour the greater part of the starch should be converted, and a watery solution of iodine should give no blue color. A reddish-violet color by iodine shows the presence of erythrodextrin. A blue or purple reaction with iodine, in a fluid that has been in the stomach one hour, indicates faulty amylolysis, due either to decreased diastase in the saliva, decreased secretion of saliva, or excessive acidity of the gastric contents.

Digestion of Proteids. — For a description of the changes pro-

duced in proteids during gastric digestion, the student is referred to the tests under Albumoses and Peptones, p. 22.

The following tests may be employed to show the progress of proteid digestion:

Native proteids, albumin, and **globulin** are precipitated by boiling the slightly acidulated solution.

Acid-albumin, or syntonin, is precipitated by carefully neutralizing the solution with decinormal sodium hydroxide as in estimating total acidity. The amount of turbidity formed in this estimation gives an idea of the amount of acid albumin present. It is best to use phenolphthalein to show the neutrality, as the solution must be exactly neutral to precipitate the acid-albumin.

Primary albumoses may be precipitated from the solution, from which the native proteids and acid-albumin have been separated, as above, by saturating the solution with $MgSO_4$ or NaCl.

Secondary albumose (deutero-albumose) may be precipitated from the filtrate from the primary albumoses, as above, by saturation with crystals of $(NH_4)_2SO_4$.

To **test for peptones**, saturate a portion of the original fluid with $(NH_4)_2SO_4$, heat nearly to boiling, and filter. To the filtrate, when cold, add phosphotungstic acid, which precipitates peptone; or apply the biuret reaction. Add to fluid an excess of NaOH or KOH and two drops of $CuSO_4$ solution. A rose-colored solution indicates peptone. In most cases this *biuret test* is all that will be needed.

The rapidity of absorption from the stomach is tested by giving the person a capsule containing 0.2 grm. (3 grs.) of potassium iodide, and then testing the saliva at the end of each minute for the iodine reaction. **With a normal stomach, iodine can be detected in the saliva ten to fifteen minutes after giving the capsule.**

The iodine is detected in the saliva as follows: Strips of filter-paper are soaked in starch-mucilage and dried. One of these papers is pressed upon the tongue, removed, and then touched with a glass rod previously dipped in some yellow nitric (nitrous) acid. The appearance of a blue spot shows the presence of iodine. Some prefer to give other substances than potassium iodide. Some use a solution of common salt of known strength, drawing out what is left in the stomach after ten minutes, and estimating the salt left in the stomach. When the capsule is given on an empty stomach, a delay in the appearance of the iodine reaction in the saliva to twenty minutes, or longer, indicates some serious organic disease of the gastric mucosa.

The motility or **motor function** of the stomach is a matter of importance, and its determination is sometimes required. When the

motility is normal or increased, the food, even if it is not digested, is passed on into the duodenum before any disturbance arises from lack of digestion. The motor function may in this way compensate for lack of digestive power in the stomach. The lack of proper motor power, on the other hand, may produce dyspeptic symptoms where the digestive power is normal, because of the long delay of the food in the stomach, with secondary fermentations. Fleischer determines the motility of the stomach by giving a gelatin capsule containing 0.1 grm. (1½ grs.) of iodoform, which drug is decomposed in the duodenum, and iodide of sodium formed, which is absorbed. Iodine can be detected in the saliva in from fifty-five to one hundred minutes when administered after the usual test breakfast.

Ewald's salol test is another chemical test of the motility of the stomach. Salol is nearly insoluble in the gastric juice, and does not enter the circulation until it is decomposed. It does not decompose until it reaches the duodenum, when it splits up into phenol and salicylic acid. A capsule containing one grm. of salol is given immediately after breakfast or dinner. The urine is tested every half hour afterwards. *Salicyluric acid* appears in the urine from sixty to seventy-five minutes after taking about 0.6 to 1 grm. of salol. Or, we may note how long the salicylic acid continues to be eliminated by the urine. If the reaction shows the acid in the urine at thirty hours or longer, it may be regarded as proof of deficient motility of the stomach. Salicyluric acid is easily detected in the urine by wetting a piece of filter-paper with this fluid and dropping on the moistened spot a drop of a ten per cent. solution of ferric chloride. The edge of the drop will assume a violet color in presence of traces of salicylic acid. These papers may be dried and preserved, if necessary, as records of the test. Klemperer pours 100 c.c. (3⅓ ozs.) of pure olive oil into the empty stomach, and removes with the stomach-tube what remains after two hours, to determine how much has been passed into the duodenum. There are several mechanical methods of testing the gastric peristalsis. They depend upon the pressure the stomach exerts upon an intragastric rubber bag attached to a manometer or other recording apparatus.

Examination of the Sediment or Solid Particles Obtained from the Stomach.—The quantity, character, and appearance of the insoluble portions of gastric contents are important. We may in this way find particles of food eaten twelve to twenty-four hours before, showing a probable dilatation of the stomach or stenosis of the pylorus. Excess of the starchy elements of the food indicates an excess of gastric acidity, or a deficiency in the activity of the saliva; while an absence of bread or starch and the presence of proteid articles of diet

indicate deficient acid. These indications will, of course, be more marked after an ordinary meal than after the usual test-meal. An examination of vomited matters will often reveal the above indications. The presence of mucus, in excessive amount, will be evident to the eye by its stringy, tenacious character. We may demonstrate its presence, chemically, by shaking the sediment with a weak solution of NaOH, filtering, and acidifying the filtrate with acetic acid, when the mucus will be precipitated. Excess of mucus is found in gastritis and gastric catarrh. Biliary coloring matters are usually evident to the naked eye, or they may be demonstrated by Gmelin's test (see p. 96), or with the spectroscope. Blood may be detected with the unaided eye when present in considerable quantity. When in small quantity, we may use the spectroscope or the guaiac and turpentine test, mentioned under Urine. The microscope, however, will usually render these tests unnecessary.

Microscopical examination should always be made of the sediment obtained from the stomach. Besides fragments of undigested food, we may recognize the staining of these particles by bile, the presence of yeast-cells, blood corpuscles, sarcinæ and bacteria of various kinds. It is best to first examine the sediment unstained, and then stained with Bismarck brown or Lugol's solution, and then with methyl-blue, which last color will reveal the bacteria to the best advantage. Bacteria of various kinds are always to be found in the stomach, and are only indicative of disease when present in very large numbers. An unusually long non-motile bacillus, first recognized by Oppler, is found in most cases of gastric carcinoma. It has the power of rapidly changing sugars to lactic acid, and in carcinoma of the stomach the number of the bacilli increases with this acid. Its absence in gastric disease is a very strong argument against a diagnosis of cancer. Excessive bacterial fermentations in the stomach usually give rise to dyspeptic symptoms, such as distention, pain, delayed or difficult digestion, disturbed intestinal peristalis, toxic absorption, etc.

THE FECES.

An adult, upon a mixed diet, passes about 120 to 180 grms. (4 to 6 ozs.) of moist excrement in twenty-four hours. A vegetable diet increases the amount. If much indigestible food be taken, it may be as much as 500 grms. The consistency depends upon the amount of water present, which is usually about from 68 to 80 per cent. A pure flesh diet gives a comparatively dry feces, while substances rich in sugar yield feces with a comparatively large amount of water. The

quantity of water taken has no fixed relation to that found in the feces. The water in the feces seems to be in direct ratio with the energy of the peristalsis.

The feces of adults have a neutral, sometimes alkaline, and rarely acid **reaction.** The alkalinity is derived from the ammoniacal fermentations, while the acidity is from lactic and butyric acid fermentations. Acetic and propionic acids have also been found in the feces. The copious secretion of mucus favors the occurrence of the neutral or alkaline reaction. The feces of infants fed upon milk are usually acid from the presence of lactic acid and free, fatty acids.

The **color** of feces depends upon the amount of altered bile-pigment mixed with them, to which the bright-yellow to dark-brown color is due, or upon the character of the food. The normal yellow-brown coloring matter of feces is hydrobilirubin. If much blood be present, — that is, after hemorrhages, — the feces may be blood red, coffee color, or brownish-black from the formation of hematin. Preparations of iron, manganese, bismuth, or lead, taken by the mouth, color the feces black from the formation of the sulphides of these metals. Some green vegetables impart a brownish-green color, due to chlorophyll. Blackberries, huckelberries and other foods may produce a black color; chocolate a gray, and santonin, rhubarb root and senna produce a yellow color. The green color of infants' feces is believed to be due to the formation of a green pigment by one or more varieties of the colon bacillus, or to butyric and lactic fermentation, and to the action of these acids upon the biliary coloring matters. In adults green passages may be due to a green microscopic organism, or **chlorococcus.**

The disagreeable *odor* of normal feces is due in great part to indol and skatol. These are products generated by the putrid bacteria, and come from the decomposition and transformation of albuminoid matters. Hydrogen sulphide, and sometimes a trace of hydrogen phosphide, ammonia and organic bases contribute to the odor of fecal matter.

Composition. — First. Alimentary substances which are assimilable, but which have been taken in excess, as starch, fatty matters in notable quantities, and a small proportion of non-assimilable proteids. Flakes of casein or paranuclein are often found in feces, but they must not be confounded with similar flakes of free fatty acids. The latter are soluble in ether.

Second. Indigestible substances, such as vegetable fiber, cellulose, chlorophyll, gums, pectic substances, resins, elastic tissue, epidermic tissue, tendons, diverse coloring matters, nuclein, chittin, insoluble

salts (silicates, insoluble sulphates, ammonium-magnesium phosphate, and calcium phosphate).

Third. Products coming from the digestive tube itself; intestinal mucus, epithelial cells, biliary acids (in traces), cholesterin, and bacteria.

Fourth. Substances which failed of absorption, as emulsified fats, the free fatty acids, leucin, and biliary coloring matters.

Fifth. Decomposition products due to microbic action, as the free fatty acids from acetic up to palmitic, this last quite abundant; also butyric, isobutyric, and lactic acids, phenol, cresol, indol, skatol, stercorin, excretin, ammonium carbonate and sulphide, urea, uric acid, xanthin bases, amins, amids, ptomaines, leucin, tyrosin, and phenylpropionic, phenylactic, and parahydroxylphenylactic acids. Some of these bodies, especially the ptomaines, acids, phenols, and coloring matters, are partly absorbed, the phenols passing into the urine in combination with sulphuric acid, as sulphuric esters.

Sixth. Pigments. The pigments found are stercobilin, hematin, biliary pigments, hydrobilirubin, and food-coloring matters.

Seventh. Gases, as CO_2, CH_4, H_2S, H_2 and N_2.

The following figures give the analysis of 1,000 parts of fresh excrement:

	Adult Man.	Infant.
Water,	733	851.3
Fixed solids,	267	148.7
Total organic matter,	208.75	157.1
Mineral matter,	10.95	13.6
Alimentary residue,	83	. . .

The analyses of excrement have their importance, principally, as a guide to the assimilation or malassimilation of food, and also because the excrement of various animals is employed as a fertilizer. Two peculiar substances are found in feces which do not occur elsewhere in the body. These are **excretin** ($C_{26}H_{36}O$) and **stercobilin,** or **serolin.** **Excretin** may be obtained from the feces by making an alcoholic extract and keeping it for a considerable time at a temperature below 0° C. (32° F.), when there is deposited an olive-colored, granular body, having a fecal odor and acid properties, from which Marcet has named it **excretolic acid.** It resembles cholesterin in its properties.

Meconium is the name given to the dark, greenish-brown, pitch-like contents of the intestine of a human fetus at birth. It is devoid of fecal odor and has an acid reaction. The microscope shows it to contain cylindrical cells from the villi, often grouped together as when covering the villi. They are usually stained green. Besides these

epithelial cells, there are cholesterin plates, fat globules, and crystals of bilirubin. Meconium contains mucin, bilirubin, biliverdin, biliary acids, cholesterin, traces of fat, and fatty acids. It contains no urobilin, glycogen, peptone, lactic acid, leucin or tyrosin. It contains from 20 to 28 per cent. of solid matter. The *feces of infants* fed with normal breast-milk are lemon-yellow to orange-colored, and contain about 85 per cent. of water, 13.5 per cent. of organic and 1.5 per cent. of mineral matter. On shaking up with water, the water becomes acid, and numerous flocculi of larger or smaller size separate.

These flakes of solid matter consist of fat, fatty acids, and lactate of calcium, with usually a very small amount of casein and epithelial cells. In some cases, the fat and fatty acids will comprise as much as 50 per cent. of the total solids, although usually it is about 1.4 per cent. of the total feces. Mucin, biliary coloring matters, and micro-organisms are usually found. While the undigested casein is usually small in amount, in infants nursed at the breast, it is usually much greater in infants fed upon cows' milk. Frequently, large masses of casein and fatty acids are seen, mixed with paranuclein.

Diarrhoeal stools, or those after taking purgative medicines, vary from the normal principally in the proportion of water, although the rapid peristalsis will usually bring down more or less undigested matters. The feces in **typhoid fever** are usually semifluid, and frequently contain blood and small portions of necrotic membrane, and usually contain crystals of ammonium-magnesium phosphate. They contain the Eberth bacillus, peculiar to this disease, and a poisonous base known as typho-toxin, having the formula $C_7H_{17}NO_2$, and an active toxalbumin. The Eberth bacillus decomposes cane-sugar, with the production of levorotatory lactic acid. **Choleraic stools** are thin and almost colorless, resembling rice-water. Under the microscope are seen a large number of epithelial cells from the villi, and bacterial analysis reveals the comma spirillum. Cholera stools contain indol and nitrites, and develop a blood-red color on adding dilute sulphuric acid, due to the formation of nitrate of nitroso-indol. This reaction is often used for diagnostic purposes. Poisonous bases and toxalbumins have also been found.

Dysenteric stools are characterized by an abundance of mucus and pus tinged with blood, peculiarly fetid, and frequently containing fragments of sloughing mucous membrane. Microscopical examination sometimes reveals an ameba, known as the **ameba coli,** characteristic of certain cases of tropical dysentery. Hemorrhages into the stomach or along the small intestine produce dark brown or black colored and very fetid stools.

Intestinal Gases. — The large and small intestines always contain more or less gas. This is usually a mixture of carbon dioxide, methane, nitrogen, and hydrogen. In strictly normal digestion, unless substances rich in sulphur are eaten in considerable quantities, sulphuretted hydrogen is not found in the gases of the colon. The CO_2 is derived in part from bacterial decomposition of carbohydrates and proteids, and in part from the blood. The CH_4 is derived from the bacterial decomposition of carbohydrates and proteids, but especially from cellulose. The nitrogen is partly derived from the blood, partly from the air swallowed, and partly from decomposition of proteids. The hydrogen is derived from fermentations of carbohydrates and proteids. Butyric fermentation of sugars generates hydrogen. The following table gives the percentage composition of the gases of the large intestine, as given by Ruge:

Vol. in 100 Vols.	Milk Diet.		Meat Diet.			Leguminous Vegetable Diet.		
	I.	II.	I.	II.	III.	I.	II.	III.
Carbon dioxide, . . .	16.8	9.9	13.6	12.4	8.4	34	38.4	21
Hydrogen, ...	43.3	54.2	3	2.1	0.7	2.3	1.5	4
Marsh-gas, ...	0.9	. .	27.5	27.5	26.4	44.5	49.3	55.9
Nitrogen, ...	38.3	36.7	57.8	57.8	64.1	19.1	10.6	18.9

Examination of Feces.—Inspection.—The proper examination of the feces should consist of an *inspection*, a *macroscopic*, a *microscopic* and a *chemical examination.* From an inspection of the feces we learn the quantity, color, consistency and odor. Sometimes the presence of undigested particles of food, seeds of fruits, curds of milk, masses of fatty acids, mucus, blood, pus, concretions, worms or their eggs may be thus found.

The *quantity* of the stool will depend upon the amount and character of the food taken. Carbohydrate foods containing much cellulose give a voluminous stool. Pure starch and sugar leave little if any residue. Fresh meats leave little residue in the normal state of digestion, but in diseased conditions the residue of meat fibers may be considerable. In breast-fed infants there is from 6 to 10 grms. of fecal matter for each pound of body weight. Bottle-fed babies pass from 20 to 50 grms. per pound of weight per day. The quantity is increased by laxatives, and especially during diarrhoeas. From the amount and character of the feces expelled we may learn much of the

digestive powers, or the suitability of the food. The average amount of feces is diminished in constipation. The *consistency* of stool is generally designated as dry or moist, formed, mushy or liquid, and watery or slimy.

Liquid, dry, watery or slimy stools are abnormal. The normal adult stool is moist, and moulded into a cylindrical shape, tenacious enough to cohere into elongated masses. The milk stools of infants are normally mushy or pasty and not formed into cylindrical masses.

Liquid stools are abnormal and indicate the use of cathartic medicines, excessive intestinal peristalsis, serous exudation from the intestinal mucosa, or some failure in normal absorption.

A formed, flat or wire-drawn stool indicates some stenosis of the rectum or anus.

The **reaction** of the feces to litmus paper may be acid, neutral or alkaline. The normal reaction of infants' stools is acid. An alkaline reaction is abnormal. It may become alkaline in digestive disturbances with proteid fermentation, in cholera infantum and colitis.

In older children and in adults the reaction may be either acid or alkaline. The acidity may be determined by rubbing up 20 to 30 grms. of the feces with hot distilled water, making the volume up to 100 c.c., filtering off an aliquot portion, say 50 c.c., and titrating the filtrate with $N/10$ NaOH, using phenolphthalien as the indicator. Blanberg found the acidity of the stools of breast-fed infants to be 25 c.c. of $N/10$ NaOH for 100 grms. of feces, and 11.33 c.c. for 100 grms. of feces of babies fed on cows' milk.

Foreign substances may best be detected by washing the stools with water, floating off the lighter particles and examining the heavier portions of the residue, best in a petrie dish. Undigested food may often be seen with the unaided eye. When fat is present in abnormal quantities, it may be recognized as whitish or grayish masses, varying from the size of a pea to that of a lima bean. These masses may be mistaken for casein. At times the fat may not be evenly mixed with the feces, but covering the entire mass like an envelope. This abnormally large amount of fat — *steatorrhoea* — is usually associated with diseases of the intestines affecting the absorptive power, as atrophy or amyloid degeneration of the mucosa, or in diseases of the glands or vessels of the mesentery, in tubercular peritonitis, tuberculosis of the mesentery, catarrh of the small intestine and in some cases of pancreatic disease. **Mucus** may be seen in catarrhal conditions of the colon and rectum. Occasionally masses are seen, drawn out into ribbons, and are often passed with or without fecal matter, and with considerable pain and tenesmus. This condition is some-

times known as *enteritis membranosa* or *colica mucosa*, and is due to a catarrh of the colon or rectum, or it may be a *secretion neurosis*.

Examination of Feces for Solid Substances. — The feces to be examined are placed in a small fine wire seive, another seive a little smaller than the first is inverted upon it and a slow stream of water from the tap is allowed to run through both seives until all soluble parts are washed through. By this method we may obtain :

Flakes of casein, insoluble fatty acids, seeds of small fruits, remnants of certain foods, as corn, berries, etc. In children and the insane we may often find stones, and other foreign substances of varied character. **Bilary calculi, enteroliths** and other solid bodies may be looked for by this method.

Microscopical Examination. — For microscopical examination the feces should be as fresh as possible. If it is desired to examine for amoebæ or other microörganisms this is very important. For ordinary examinations it is best to shake some of the fecal matter in a flask or test tube with water and, after standing a moment, pour off the turbid supernatant liquid and let it settle and examine this sediments separately. The heavier particles will thus be washed by repeated treatments with clean water.

When examined with a microscope of moderate power there will usually be found particles of food, the epidermis of cereals and vegetables, threads of cellulose, seeds, etc. By this means we may often determine what foods or food constituents are undigested. An abnormal increase of muscle fiber, starch, or fatty acids, generally indicate a severe catarrhal condition of the small intestine.

The epithelial cells, mucus and small remnants of vegetable foods are, in normal conditions, usually stained yellow by biliary coloring matters.

Casein and *paracasein* frequently occur in the stools of children and in adults taking milk. To distinguish casein from fatty acids it is only necessary to treat the suspected lumps with ether, or petroleum ether, when the latter dissolve, while the former do not.

Leiner's test may also be used as follows: A small portion of the feces is spread out on a slide, dried and fixed by passing the slide three or four times through the Bunsen burner flame. It is then stained with a mixture of equal volumes of 0.75 per cent. alcoholic solutions of acid fuchsin and of methyl green, diluted with ten volumes of water. After staining fifteen minutes, wash well by immersion for an hour in distilled water. Casein and paracasein are stained blue or violet. The fatty acids do not stain at all.

Fat occurs in feces in the form of neutral fat in round highly

refracting drops, also needle-shaped crystals, of fatty acids, or of soaps of the higher fatty acids with calcium and magnesium.

Cholesterin plates are often found. Starch grains may be mistaken for fat droplets, but they are easily differentiated with iodine, with which they give a blue color.

Neutral fats stain red or bluish-brown with tincture of alkanet. Osmic acid stains both free fat and fatty acids black. The amount of fat in stools varies greatly in different individuals. The quantitative estimation therefore has little practical value.

The fatty acid crystals melt when heated, and are soluble in cold alcohol or in a weak solution (5 per cent.) of sodium hydroxide, while the neutral fats are insoluble in both. Both the neutral fats and fatty acid crystals are soluble in ether and chloroform.

The soaps of calcium and magnesium occur as amorphous masses and as crystals. They are insoluble in hot water, alcohol and ether. The insolubility in alcohol and ether serve to distinguish them from the fatty acids and from neutral fats. Neither osmic acid nor tincture of alkanet stain them.

The estimation of fat and fatty acids may be accomplished by extraction of the dried feces with ether, evaporating off the ether, washing the residue with cold alcohol to dissolve out the fatty acids and cholesterin and drying and weighing the neutral fat.

The fatty acids may then be treated with a standard alkali in excess, and the excess of alkali estimated by back titration with a standard acid.

Crystals. — Beside the crystals of fatty acids and soaps, above referred to, crystals of ammonium magnesium phosphate are often found. They are similar in form to those found in urine, and are readily soluble in dilute acetic acid.

Charcot-Leyden crystals occur occasionally in normal stools. They are colorless, transparent, elongated octahedra. They are quite constantly associated with the presence of intestinal worms. Their presence in the feces should excite suspicion of the presence of these parasites. Their persistence after treatment for tapeworm is strong evidence that it has not been entirely expelled.

Anatomical Elements. — For the examination for cells and bacteria the object should be prepared as follows: A small portion of the feces should be spread out on a slide and dried. This is fixed by passing it through the flame two or three times, then washed with ether, alcohol, then with a few drops of dilute acetic acid and finally with water. This specimen may be examined with or without staining. Mucous masses will usually be found, containing bacteria and a few

epithelial cells. In intestinal diseases these masses will be more abundant, and filled with numerous leucocytes, and degenerated epithelial cells, or possibly red blood cells.

Epithelial cells when present in large numbers indicate an inflammatory condition of the mucosa of some part of the intestine. When stained with bile, they are from the small intestine. They are usually associated with an increase of mucus, whatever be their origin. Cancer cells may be found in carcinoma of the rectum.

Blood cells will only be found when the seat of the hemorrhage is in the descending colon, rectum or anal orifice. If the hemorrhage is higher up the coloring matter of the blood is changed to hematin or hematoidin. The spectroscopic examination or the test for iron, described under blood, may be used.

Mucus particles, appearing as small hyaline bodies, are indicative of catarrh of the intestine. In this case they are often bile-stained. If colorless, they probably originate in the lower part of the small or upper part of the large intestine. When mucus comes from the lower part of the colon it is not well mixed with the feces. When the mucous masses are stained yellow with bile, it is an indication of the existence of disease of the small intestine. This is especially true when they are accompanied by large numbers of cylindrical epithelial cells.

Leucocytes. — The presence of a large number of leucocytes indicates a severe catarrhal inflammation or ulcerative process in the intestine. Large amounts of pus are found in dysentery and in the rupture of abscesses into the intestine.

BILIARY CALCULI.

Biliary calculi may vary in size from that of a mustard seed to that of a filbert. In color they vary from white to a greenish black. In sp. gr. they may be lighter than water or they may be heavier. In composition they may consist chiefly of cholesterin, of inspissated bile, or of calcium salts. Those of the first class are light colored and float on water, and are soft. Those of the second class are harder, brown or very dark green in color, and are heavier than water. Those composed chiefly of lime salts are generally irregular in shape. All forms of these stones are liable to be somewhat mixed in composition. There may be a nucleus of calcium phosphate or sulphate, and the rest of the stone may be organic. These stones frequently present facets formed by contact with one another during their formation in the gall bladder.

Chemical Analysis of Gall Stones. — 1. The stone is pulverized, and about one or two grms. of the powder are treated in a flask or

test-tube with about 10 to 20 c.c. of ether, shaken a few times and filtered through a dry filter.

2. The ether solution, containing the most of the cholesterin and fat, is allowed to evaporate. The residue is treated with hot alcohol and a drop of this alcoholic solution is allowed to evaporate spontaneously on a microscopic slide and the residue examined by a microscope for the rhombic plates of cholesterin, $C_{27}H_{46}O$.

3. The residue, insoluble in ether, is washed once on the filter with ether, and the residue is treated with dilute HCl (1 : 3). The acid should be returned to the filter and run through about three times. The calcium salts are thus dissolved, along with a trace of copper. The calcium may be detected by adding NH_4OH in excess when the phosphate precipitates. This precipitate may be filtered off, and ammonium oxalate added to the filtrate to precipitate any remaining calcium. A few drops of K_4FeCy_6 may be added to a portion of the acid solution, when a brown color or precipitate will indicate copper.

4. The residue insoluble in HCl is washed with water until the washings are free from HCl, as shown by $AgNO_3$, dried, and the residue separated from the paper; or, the paper is cut up into small pieces and warmed in a dry flask with chloroform. Bilirubin ($C_{32}H_{36}N_4O_6$) dissolves, giving a brown yellow solution. Filter. Shake a portion of the filtrate with a weak solution of Na_2CO_3, when the color is taken up by the alkaline solution. On standing, this solution absorbs oxygen and turns green from the formation of biliverdin, $C_{32}H_{36}N_4O_8$. Occasionally *concretions* are found in feces, which have a nucleus of hardened feces covered with a crust of calcium and magnesium salts and known as *enteroliths*. In herbivorous animals these bodies often contain a nucleus of hair, and attain a large size.

MILK.

Milk is the secretion of the mammary glands, the presence of which is characteristic of the mammalia. The milk of different animals differs somewhat in composition, but always contains the same constituents. It contains all the necessary constituents of a perfect food, and is intended for the nourishment of the young until they are able to live upon other foods. Milk is an opaque, white fluid, containing fat globules in suspension, albumin, sugar, and salts in solution, and caseinogen in partial solution. The reaction of human milk is generally feebly alkaline; that of the cow is usually neutral or faintly acid, while that of the carnivora is generally acid. Milk readily becomes acid or sour on exposure to the air, due to lactic fermentation.

Microscopical examination reveals the fat in a state of perfect emulsion, the fat globules remaining suspended and separate from one another. The fluid seen between the globules is not perfectly clear, but contains small granules of caseinogen, which may be filtered out by passing it through a clay filter.

Colostrum. — The milk which is secreted for the first few days after parturition is viscid, yellow in color, of high specific gravity, and shows the presence of a few corpuscles of a peculiar character, called *colostrum corpuscles*. These corpuscles seem to be epithelial cells containing fat globules, which they have not yet liberated. The composition of colostrum varies in different individuals. The total solids vary from 13 to 15 per cent.; the caseinogen, 1.5 to 3 per cent.; albumin and globulin, 1 to 7 per cent.; sugar, 3 to 6 per cent.; and salts, 0.3 to 0.5 per cent. Colostrum is coagulated by heat, while ordinary milk is not. It is often not coagulated by rennet. It is curdled by acetic acid, by $HgCl_2$ and by alcohol.

The **specific gravity** of milk is usually determined with the hydrometer. The specific gravity of cows' milk varies from 1029 to 1035. An excess of fat lowers the specific gravity and the removal of fat raises it. The addition of water will lower the specific gravity. These facts are made use of for the detection of the ordinary adulterations of milk.

The amount of milk secreted varies with the health of the animal, the amount of food taken, and various other conditions. The amount secreted by a woman each day is about 1 liter. A good cow secretes about 8 to 12 liters. The composition of milk varies in different classes of animals, with the state of nutrition of the animal, the constitution, the age, the period of lactation, and the character of the food.

Composition of Milk. — The published analyses of milk are very numerous, and the older analyses, made by older methods, differ somewhat from the analyses made by more recent methods. The analyses differ so much that it is evident that milk is subject to considerable variation in composition and it is impossible to give exact figures representing its composition. The comparative composition of human milk and of cows' milk is given in the following table, as nearly as possible.

Under the heading of "extractive matters" is included a considerable number of substances occurring in very small quantities, among which are creatin, leucin, the odoriferous principle, lecithin, nuclein, etc. Among these lecithin and nuclein are the most important from a nutritional point of view. It also usually contains certain gases,

AVERAGE ANALYSIS OF HUMAN MILK AND COWS' MILK.

	Human Milk.			Cows' Milk.		
	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.
Water.	83.69	9.09	87.75	80.32	91.50	87.13
Total solids.	9.10	16.31	12.26	9.00	19.68	13.67
Specific gravity.	1028	1033	1032	1029	1033	1031.4
Fat.	2.00	7.6	3.13	3.00	9.82	3.79
Sugar (lactose).	6.00	7.00	6.78	4.00	5.co	4.90
Caseinogen.	0.35	1.5	0.5	2.70	4.50	2.88
Lactalbumin.	0.75	1.6	1.1	0.20	0.80	0.60
Citric acid.	0.05	0.05	.05	0.05	0 15	0.05
Extractive matters.	0.35	0.55	.50	0.45	1.00	0.75
Ash.	0.20	0.30	.20	0.65	0.78	0.70

principally carbon dioxide, oxygen, and nitrogen. Colostrum contains a larger proportion of solid matter than ordinary milk, a larger amount of proteids, and less sugar.

The table on next page shows many differences between human and cows' milk not shown in the above table, and which are of importance in adapting the latter to infant feeding.

Milk Proteids.—The proteids which occur in milk are probably at least four in number—*caseinogen*, *lactalbumin*, *lactoglobulin* and *albumose*. When milk is allowed to stand at the ordinary temperature a part of its lactose is converted by fermentation into lactic acid. When this has accumulated to a considerable extent the caseinogen is precipitated. When milk is treated with rennin, or gastric juice containing this ferment, the caseinogen is rapidly converted into casein, and at the same time coagulation or precipitation occurs. When rennet is added to cows' milk, the result is a coherent clot or curd and a clear, yellowish fluid called whey. The curd contains the fat entangled with the casein. The whey contains the albumin, sugar, and salts. In human milk the curd is formed of smaller flocculi, and the same appearance may be produced with cows' milk if it be previously boiled or largely diluted with lime-water. Human milk usually contains more albumin than caseinogen, a fact that is of great importance in the behavior of the milk with rennin or with acids—*i. e.*, in the first stages of digestion. This relation between the caseinogen and albumin determines very largely the character of the curd produced on coagulation. The larger the proportion of albumin to the caseinogen, the finer the flocculi formed. The actual amount of proteids in human milk varies at different periods of lactation, being greatest during the first week. The following figures

TABLE SHOWING DIFFERENCES BETWEEN HUMAN AND COWS' MILK.

PROPERTIES.	HUMAN MILK.	COWS' MILK.
Physical appearance.	Bluish, translucent, odorless, sweetish.	Opaque, white or yellowish-white, distinct odor, feeble sweet taste.
Specific gravity.	1026 to 1036.	1029 to 1035.
Reaction.	Amphoteric or slightly alkaline.	Amphoteric or slightly acid; becomes quickly acid on exposure to air.
Behavior on boiling.	Does not coagulate, and forms a very slight pellicle, scarcely observable.	Does not coagulate, but forms a distinct pellicle of casein and lime-salts.
Spontaneous coagulation.	Coagulates only after one to two days, at room temperature.	Coagulates after six to twelve hours at room-temperature; due to lactic acid.
Coagulation with rennet.	Coagulates incompletely in small, isolated flocculi, never forming visible curds.	Coagulates at body temperature, separating into curdy masses and opalescent whey.
Fat.	But er yellowish, similar to cow-butter; sp. gr. at 15° C. = 0.966; melts at 34°C.	Butter yellow-white; sp. gr. at 15° C. = 0.949 to 0.996; melts at 35.8° C.
Composition of fat.	Olein, palmitin, stearin, butyrin, caprin, caproin, myristin.	Olein, palmitin, stearin, caproin, caprylin, caprin, laurin, myristin, arachin, butyrin, lecithin, cholesterin.
Relation of acids.	Volatile acids relatively small; oleic acid, one-half non-volatile acids.	Volatile acids relatively large, oleic acid small, palmitic and stearic large.
Caseinogen.	Precipitated with difficulty by salts and acids; easily soluble in acids; leaves no pseudonuclein with peptic digestion.	Precipitated easily by salts and acids; precipitate not easily soluble in excess of acids; leaves residue of pseudonuclein.
Composition of proteids.	Lactalbumin, lactoglobulin, and casein; ratio of casein to albumin, 1 to 1.2 (?).	Lactalbumin and globulin small; casein to albumin, 1 to 6 to 1 to 7.
Mineral matters.	Relatively small amount; contains one-sixth as much CaO and one-fourth as much P_2O_4 as cows' milk.	Contains relatively large amount.
Nuclein and lecithin.	Nuclein, 1.2 gm. per liter (Siegfried); lecithin, 1.7 gm. per liter (Stoklasa).	Nuclein, 0.6 gm. per liter (Siegfried); lecithin, 1 gm. per liter (Stoklasa).
Bacteria.	Generally sterile; rarely staphylococcus albus and aureus.	Contains numerous bacteria, and occasionally typhoid, diphtheria, tubercular organisms, etc.

show this variation, and the relative percentage of albumin and caseinogen (Camerer) :

Period of Lactation.	Total Proteids.	Caseinogen.	Albumin and Globulin.
Eighth to eleventh day,	2.53	0.91	1.62
Twentieth to fortieth day,	1.74	0.55	1.19
Two to three months,	1.46	0.46	1.00
Eight months,	1.19	0.35	0.84

From the above analysis we see that the amount of totalproteids in human milk during the first two weeks of lactation is about 2.5 per cent., and in many cases 3 per cent., but of these nearly two thirds are soluble albumin and globulin instead of caseinogen.

The term *casein* is sometimes restricted, as above, to the precipitated proteid formed by the action of rennet upon the caseinogen of milk. Casein is the chief constituent of cheese.

Lactalbumin.—After the precipitation of the caseinogen by acetic acid, this proteid is left in the solution. The scum which forms on the top of boiling milk is probably formed by the coagulation of a part of the lactalbumin and caseinogen.

The boiling of milk before it is used as food is advantageous, in that all germs of disease are destroyed, and that the rennet gives a flocculent instead of the heavy, curdy precipitate; it is objectionable for a number of reasons. The lactalbumin is modified by the boiling, and precipitates on adding acid to the cold boiled milk.

Lactoglobulin.—Various observers have discovered other proteids than the two mentioned above. Lactoglobulin, whey proteid, lactoprotein, proteoses, peptones, and nuclein have been mentioned as occurring in milk, but our knowledge of the amount present is slight. Van Slyke, and Babcock and Russell claim that cows' milk contains from 0.3 to 0.5 per cent. of albumoses and peptone. It is claimed by some that their supposed presence is due to faulty methods of examination. *Lactoglobulin* has the property of liquefying cooked starch and of partially digesting it. This property is destroyed by boiling.

Fat.—The fat in milk exists in the form of microscopic globules, varying from 0.01 to 0.0016 mm. in diameter, or in the form of a true emulsion. Whether each globule is surrounded by a membrane is yet an undecided point. The weight of opinion is in the negative. The chemical composition of milk-fat is very nearly like that of adipose tissue, with small quantities of the triglycerids of butyric, caproic, caprylic, myristic, and arachidic acids. Milk also contains minute quantities of lecithin, cholesterin, and yellow lipochrome.

Cream is simply the upper layers of milk which has been left to stand, and in which the fat globules are more numerous than in whole milk. The amount of cream that will separate from the milk in twenty-four hours is sometimes made use of as a test for the richness in fat. A fair market milk will give from 10 to 12 per cent. of cream, while rich milk will frequently give from 15 to 20 per cent., or even more.

Market cream is of two kinds: **Gravity cream** is that separated by allowing the milk to stand, and skimming off. Gravity cream contains from 10 to 28 per cent. of fat, varying with the amount of fat and proteids in the original milk and the conditions of the creaming.

Centrifugal cream is that separated by a centrifugal machine, and can be prepared of almost any per cent. of fat.

Butter is the fat of milk in which the fat globules are separated from the other constituents by mechanical agitation in a churn. About one sixth of the fat remains in the buttermilk. Buttermilk contains, therefore, about from 0.5 to 1 per cent. of fat. Butter also contains small quantities of caseinogen and lactose. Butter from human milk is richer in fluid fats than that made from cows' milk. By exposure to the air butter becomes rancid, due to the saponification of some of the glycerids of the fatty acids.

Milk-sugar, as will be seen in the above table, occurs to the extent of about 4.9 per cent. in cows' milk, and about 6.7 per cent. in human milk. Recent investigations seem to show that the sugar of cows and human milk are not the same.

The **salts** of milk are the phosphates of potassium, sodium, calcium, and magnesium, with chlorides of potassium and sodium, and a trace of iron, which is in combination in the nuclein. The most of the phosphorus of human milk and about one half that of cows' milk exists in organic combination in the nuclein and lecithin.

QUALITATIVE EXPERIMENTS WITH MILK.

1. Examine a drop of milk with the microscope. Allow some milk to stand for three hours, and siphon off the lower three-fourths with a rubber tube. Use the skim-milk thus drawn off for the tests.

2. Note the specific gravity of fresh milk with the lactometer, and observe that the specific gravity is increased by the removal of the lightest constitutent — the cream. Compare the specific gravity of the rich top-milk with the skim-milk drawn off.

3. Test the reaction with sensitive litmus paper. The reaction of fresh human milk is usually neutral or slightly alkaline. The reaction of fresh cows' milk is neutral or slightly acid to litmus.

4. Warm some milk in a test-tube, to the temperature of the body, and add a few drops of *liquid rennet* or *essence of pepsin.* After standing, a *curd* is formed from the conversion of *caseinogen*, the chief proteid in milk, into *casein.* The casein entangles the fat globules; the liquid is termed *whey.* No curd forms if the rennet solution is previously boiled. Heat kills ferments.

5. To another portion of warm milk diluted with water, add a few drops of strong acetic acid. A curdy precipitate entangling the fat is formed.

6. Filter off the curd, and test the whey for *lactose* or *milk-sugar*, by Trommer's test; for *lactalbumin* by boiling, or by Millon's reagent; and for earthy (that is, calcium and magnesium) *phosphates* by ammonia, which precipitate these phosphates.

7. *Fat* (*butter*) may be extracted from the curd by shaking it with ether or petroleum-ether; on evaporation of the ethereal extract the fat is left behind, forming a greasy stain on paper.

8. *Caseinogen*, like the globulins, is precipitated by saturating milk with sodium chloride or magnesium sulphate, but differs from the globulins in not being coagulated by heat. The precipitate produced by saturation with salt, floats to the surface with the entangled fat. The clear, salted whey may be obtained by filtering off, after standing fifteen minutes.

9. *Experiments on Coagulation of Milk.* — Prepare a solution of pure caseinogen as follows: Saturate milk with magnesium sulphate, by shaking it with excess of the powdered salt. Allow it to stand for a few hours and then filter. The caseinogen and fat remain together on the filter. Save the filtrate and label it A. Wash the precipitate on the filter with a saturated solution of magnesium sulphate, until the washings contain no albumin. Add water to the precipitate. The caseinogen dissolves, the fat being insoluble. In this way a solution of caseinogen in weak magnesium sulphate is obtained. So far the operations should be performed beforehand by the demonstrator.

10. To this solution add acetic acid. The caseinogen is precipitated; collect it on a filter; wash the acid away with distilled water. Dissolve the precipitate by grinding it up in a mortar with lime-water; filter, and an opalescent solution of caseinogen is obtained.

11. To a portion of the solution in lime-water add a few drops of rennet extract. Put it in the water-bath at 40° C., and, if the caseinogen has been thoroughly washed, no coagulation will occur.

12. Treat another portion in the same way, adding, however, a few drops of 0.5 per cent. phosphoric acid as well as the rennet.

Warm to 40° C. Coagulation — that is, formation of casein from caseinogen — usually occurs in a few minutes.

13. Examine the filtrate A (see 9). Saturate a portion with sodium chloride. A small amount of precipitate of a proteid precipitates. This is the so-called lacto-globulin. Milk contains only a trace of true globulin. The precipitate is mostly caseinogen previously left in solution, together with calcium sulphate.

14. Heat another portion of filtrate A to 77° C., acidifying faintly with a few drops of 2 per cent. acetic acid. Lactalbumin is coagulated at this temperature.

15. Prepare some whey as under 4, and heat it to 70° C. Mix together three volumes of the whey and one volume of the rich top-milk prepared in 1. Dissolve in this mixture milk-sugar in the proportion of 0.5 grm. to 25 c.c. Compare this mixture with whole milk, and with milk diluted with twice its volume of water, by tests 4 and 5, above. Compare it with human milk.

16. Boil thoroughly about 1 grm. of barley flour with 25 c.c. of water, and mix with an equal volume of whole milk. Compare with whole milk, and with a mixture of equal volumes of milk and water, and with the mixture prepared in 17, when treated with rennet and with acid. Observe the appearance of the curd.

17. Filter some milk through a clay filter by means of a Bunsen's filter-pump. Fit a doubly perforated rubber stopper into the open end of a porous battery-cup. Fit two glass tubes into the stopper, one closed by a pinch-cock and the other connected with the pump. Set the cup into some milk in a beaker and apply suction. After a time the cup will contain a clear milk-serum, or whey, containing lactose, lactalbumin, and salts. Caseinogen and fat do not pass through. Test the clear fluid for proteids, lactose, and salts.

The Adulterations of Milk and Milk Standards. — The adulterations usually practiced are the extraction of cream, or the addition of water, or both. Occasionally the addition of some foreign substance, as sodium carbonate, formalin, common salt, or sugar, is met with.

The detection of the adulterations of milk usually depends upon the determination of the specific gravity, the fat, total solids, and the ash. The quantity of these ingredients is not perfectly uniform, and hence certain limits of allowable variation have been determined upon from time to time. The standard adopted in many states in this country is a specific gravity not less than 1029 and total solids not less than 12 per cent., of which 3 per cent. shall be fat. The legal limits for total solids vary from 12 to 13 per cent., and the solids not fat from 8.5 to 9.5 per cent. The Society of Public Analysts of Great Britain

have adopted for total solids, 11.5; fat, 3; and solids not fat, 8.5 per cent.

For ordinary purposes the estimation of the total solids, the fat, and the ash are considered sufficient to determine the question of the adulterations usually met with in the market. The standards that have been fixed by law in a number of the states all refer to specific gravity, fat and total solids. Prosecutions are, therefore, usually based on these data. To calculate the percentage of pure milk in a mixture, the following formula may be adopted, based upon the legal standard of the State of New York — viz., 12 per cent. of milk solids, 3 per cent. of fat, and 9 per cent. of solids not fat:

$$9 : \text{solids not fat} :: 100 : x = \text{milk used in making 100 parts of the mixture.}$$

For other standards the first member of the equation will be the legal percentage of solids not fat. When the solids not fat are less than 9 per cent., it indicates some form of falsification. Suppose, for example, the solids not fat in any given analysis were 8.1. Substituting this in the above proportion, we have:

$$9 : 8.1 :: 100 : x = 90;$$

or, this sample of milk had been made from 90 per cent. of milk and 10 per cent. of water. If the milk is skimmed, the percentage of fat removed can be ascertained by the following formula:

$$\tfrac{3}{9} \times S - F = x;$$

in which S = solids not fat, and F = fat found. Suppose, for example, the fat in a given case be 2 per cent. and the solids not fat 8 per cent. Substituting these in the above equation, we have:

$$\tfrac{3}{9} \times 8 - 2 = x = 2;$$

that is, 2 per cent. of fat has been removed from this milk.

Milk Testing. — There is no instrument of simple construction which will with certainty detect the presence of a small amount of adulteration in milk. The **lactometer, or lactodensimeter,** which has been employed very largely in the sanitary inspection of milk, is a hydrometer with a scale covering the variations usually met with in milk. (See Fig. 42.) The **lactometer** of the New York Board of Health is a hydrometer on which the scale is so constructed that 100°

indicate a specific gravity of 1029, the supposed lowest specific gravity of pure milk. The space between 1000, the specific gravity of water, and 1029 is divided into 100 arbitrary degrees. The scale is extended to 120°, which corresponds to a specific gravity of 1034. When taken alone, it is of very little value. If, however, it be taken with the estimation of either the total solids or the fat, it is of considerable service. In very exceptional cases the milk of a single cow may have a specific gravity below 1029, but such milk should be regarded as abnormal.

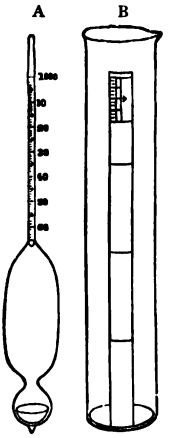


FIG. 42. — A. HYDROMETER. B. CREAMOMETER. — (*Starr.*)

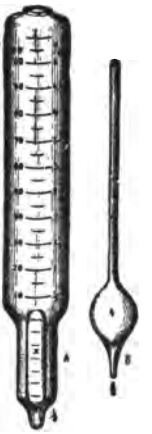


FIG. 43. — FESER'S LACTOSCOPE. — (*Queen.*)

Such depression of the specific gravity never occurs in the mixed milk of several well-fed cows. A specific gravity below 1029, therefore, unless accompanied by an excessive amount of fat, may be taken as evidence of contamination, probably with water.

The fat for such examinations may be estimated by the creamometer, or by some form of lactoscope. The creamometer, or cream gage, is simply a graduated cylinder, the graduations being $\frac{1}{100}$ of the total capacity of the cylinder to the zero mark. (See Fig. 42.) The milk is added in the cylinder to this zero mark, and allowed to remain at rest for twenty-four hours, when the number of the divisions covered by the cream is read off. This should not be less than ten per cent. The **lactoscope** depends upon the assumption that the opacity of the milk is proportional to the amount of fat which it contains.

Feser's lactoscope (Fig. 43).— In using this instrument a measured volume of milk is placed in a graduated vessel, A, by means of the pipette, B. It is then diluted with water until the black lines on the inner cylinder of opaque white glass can be seen through the layer of the mixture between the walls of the inner and outer cylinders. It is then only necessary to read off the percentage of fat on the scale of the outer cylinder at the surface of the liquid. This method of determining the fat in milk, although answering for the purpose of municipal inspection, is not to be depended upon for scientific purposes, or as evidence upon which to base legal proceedings. The lactoscope is of doubtful value in estimating the fat in human milk. In a large experience with this instrument the author has seldom seen the readings vary more than 0.3 per cent. in cows' milk, from the accurate methods. Usually it is much nearer than this.

Other simple methods of milk analysis have been devised, which serve to obtain a fair estimate of the composition of a milk, without claiming to give accurate results. These methods serve to determine whether a careful chemical analysis is necessary.

Estimation of Fat and Solids by the Densimetric Method. — This method depends upon the fact that when milk is filtered through paper the most of the fat remains upon the filter, while the other solids filter through. The specific gravity of the filtered milk is higher than the original milk. When 200 c.c. of milk are used and the portion filtering through during the first 15 minutes is taken Richmond found: (1) *If the sp. gr. of the filtered milk less 1 be divided by .004, the result will represent the solids not fat.* (2) *If the difference in sp. gr. between the original milk and the filtered milk be divided by .0008 the result will represent very nearly the per cent. of fat.* The accuracy of these rules when applied to human milk has not been investigated, but it is probable that they are nearly correct.

Example. — The sp. gr. of a milk before filtration was 1031.5 and after filtering 1034.5. The difference was .003 and the fat was found by dividing this by .0008, which gives 3.75 per cent. of fat. The solids not fat were found by dividing .0345 by .004 which gives 8.310 per cent. The total solids in this milk are, then, 12.06 per cent. The results are usually within 0.2 per cent. of the truth.

Calculation of Fat from Total Solids and Gravity. — This method is useful when great accuracy is not demanded, and may be used in examinations of cows' milk. It rests upon the assumption that one per cent. of milk solids not fat raises the specific gravity by a definite amount, while one per cent. of fat lowers it by a definite amount.

An accurate determination of the specific gravity and total solids will, therefore, furnish the necessary data for calculating the amount of fat. The formula by which the calculations are made is that of Richmond. It is as follows: $T = G/4 + 6/5F + .14$ in which G is the sp. gr., T total solids, and F the fat.

The specific gravity must be determined with care, and for rapidity only 5 c.c. of milk are evaporated to dryness to determine the total solids. This may be conducted in a watch-glass, using 2 or 3 c.c., if a platinum dish is not at hand.

To illustrate the use of this formula let us assume, in the examination of a given milk, that the specific gravity, or G, was found to be 1032 and that the total solids, on examination, gave 12 per cent. Substituting these figures in the above formula, we have:

$$12 = \tfrac{32}{4} + \tfrac{6}{5}(1.2)F + .14.$$

Then,

$$\tfrac{6}{5}F = 12 - (8 + .14) \cdot = 3.86$$

and

$$F = 3.86 \div 1.2 = 3.21 \text{ per cent. fat.}$$

To calculate the solids not fat from the specific gravity and fat, Babcock divides the sp. gr. by four, and adds to this one fifth of the per cent. of fat.

For example: The sp. gr. of a sample was found to be 1030.8 and the fat 4 per cent. $30.8 \div 4 = 7.7$; $\frac{1}{5}$ of $4 = 0.8$. Then by the rule the solids not fat would be $.8 + 7.7 = 8.5$ per cent.

The Detection of Preservatives. — The great liability of milk to become sour, when not properly cared for, has often led to the addition of preservatives to prevent this. Those most used are boric acid, salicylic acid, and in recent years formaldehyde, which is added in the form of a 40 per cent. solution, known in the market as "*formalin,*" "*formal,*" "*formol,*" "*formine,*" etc.

The most generally used preservative at the present time is formaldehyde. The most characteristic test is **Hehner's test.** The milk is diluted with an equal volume of water, and this mixture is floated upon strong H_2SO_4 in a test-tube. The presence of formaldehyde is shown by a violet-blue color at the junction of the two liquids, which is permanent for two or three days. In the absence of formaldehyde, milk gives with H_2SO_4 a greenish tinge, and on standing a brown color develops.

The addition of a few drops of ferric chloride solution to the acid makes the test more sensitive. This test is delicate and characteristic, detecting 1 part of formaldehyde in 200,000 parts of milk.

Schiff's reagent may be used as a confirmatory test. This reagent is made by just decolorizing a solution of fuchsin with sulphurous acid, without adding excess of this acid.

The milk is curdled with dilute H_2SO_4, the curd filtered out, the above reagent added to the whey, the tube corked and let stand.

When formaldehyde is present a pink color appears after a few minutes.

Many other tests have been proposed, but they offer no advantages over the above.

Hydrogen peroxide, previously neutralized by shaking it with $CaCO_3$, may be used as a preservative of milk. Three c.c. of the usual 3 per cent. solution is said to preserve a liter of milk for a week at room temperature.

Accurate Estimation of Total Solids and Water. — About two grms. of the well mixed sample are weighed out into a watch glass or platinum basin, and spread out into a thin layer by tipping the watch glass or basin. This is evaporated to dryness on a water bath, transferred to a water oven and dried to constant weight, or until it ceases to lose more than one milligramme in a half hour. The loss of weight of the milk, on drying, is calculated as water. The residue is calculated as total solids.

FIG. 44

The drying may be facilitated by adding about two or three grms. of fine washed asbestos to the platinum basin, igniting and weighing. Three to five c.c. of the milk are then poured over the asbestos, the dish and contents re-weighed and dried as above.

The ash is determined by igniting the solids in the platinum basin at a dull-red heat, over a Bunsen burner, until the ash is white. Cool in a desiccator and weigh. The difference between this weight and that of the basin and asbestos is calculated as ash.

Estimation of the Fat. — When extreme accuracy is not required, as in the examination of human milk for clinical purposes, the fat may be determined in the residue on the asbestos, after the estimation of the total solids by the above process.

The residue is treated with pure ether, warmed, allowed to stand a few minutes and poured off through a small dry filter into a previously weighed beaker, repeating this process until the fat is completely dissolved out, which will usually require about 50 c.c. of ether. The ether is allowed to evaporate, and the fat which remains behind is weighed; or, the residue left in the dish may be again weighed, when the difference between this weight and the weight of the dry solids

will give the amount of fat. The results are about 0.2 per cent. too low.

The ash may now be determined by igniting the residue left after treatment with ether, at a dull-red heat, until all the organic matter is burned off, weighing the residue and calculating it as ash.

The Centrifugal Method. — This method of separating the fat from milk for analytical purposes is quite satisfactory. The centrifugal machine has, in recent years, come into common use both in the chemical laboratory and the physician's office.

In Babcock's method 17.6 c.c. of the milk are mixed with 17.5 c.c. of concentrated pure H_2SO_4 (sp. gr. 1831 to 1834) in a special bottle provided with a long narrow graduated neck (Fig. 44). In pouring the acid and milk from the pipette, which holds exactly 17.6 c.c., into the bottle, the latter is held in an inclined position and the liquid is allowed to run down the side of the neck to allow the air to escapə by the side of the stream. After adding the acid to the milk, the two liquids are well mixed, by giving the bottle a rotary motion, until the curd is entirely dissolved by the acid.

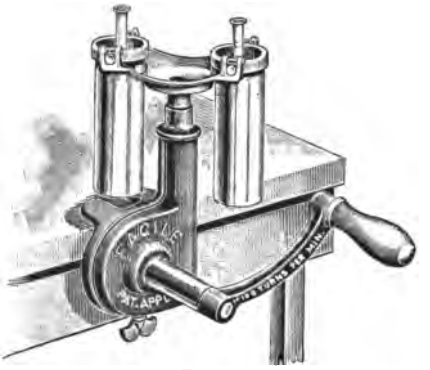


FIG. 45.

The bottle is now placed in the receiver of the centrifugal machine, as shown in Fig. 45, the receiver filled with boiling hot water and the machine rotated for five minutes. It is then allowed to slow down and stop. Boiing water is added to the bottles to bring the fat up into the graduated neck of the bottles. The machine is again rotated for one minute.

The fat will be found as a clear layer of melted fat in the neck, and the per cent. read off by the number of divisions of the scale occupied by the layer. Each small division of the scale represents 0.2 per cent. of fat. The estimations are made in duplicate, when the amount of milk at hand is sufficient. When not, the other bottle and receiver are filled with water to balance the machine. The results are quickly obtained and are accurate.

Leffman and Beam have modified the process by adding to the milk a small quantity of a mixture of amyl-alcohol and hydrochloric acid, before adding the sulphuric

acid. The size of bottle, the quantity of milk taken, and the centrifugal machine used is somewhat different from those of Babcock.

More recently, this method has been modified by Lewi so as to adapt it to the small centrifugal machines used by physicians (Fig. 10).

The process of making a determination of fat by this method is as follows: Fill the pipette to the mark with the milk to be examined. This represents 2.92 c.c. of milk. Introduce the end of the pipette carefully, so that the end is well into the body of the bottle, before delivering the milk. Cleanse the pipette, fill it with pure, strong sulphuric acid, and pour this into the milk in the bottle, with the same care as before. Add 0.6 c.c. of a mixture of equal parts of amyl-alcohol and pure concentrated hydrochloric acid. Mix the contents of the bottle by a rotary motion and then fill the bottle to A with a freshly prepared mixture of equal parts of sulphuric acid and water. Two bottles are thus prepared and placed in the machine and rotated two or three minutes, when the fat will have separated and the percentage may be read off on the graduated neck of the bottle. The space between the smaller lines represents 0.3 per cent. of fat. To insure greater accuracy, it is best to use a small hand magnifying glass, with the aid of which ½ or even ¼ of a division may be read, and thus indicate 0.1 per cent.

This process has no advantages over the Babcock, except the small amount of milk required. It has the disadvantage of being less accurate because of that fact.

The Author's Method. — The author has used the following process for the estimation of fat, which he has found by comparison with the Babcock method to give accurate results, and it requires but 10 c.c. of milk.

Pour 10 c.c. of the well-mixed sample into a burette with glass stopcock, which will hold at least 30 c.c. To this add one or two drops (not more) of about a ten per cent. solution of NaOH, mix well and then add 10 c.c. of pure alcohol-free ether. Cork the open end of the burette and shake the mixture thoroughly until a uniform emulsion is obtained. Now add 10 c.c. of pure methyl alcohol (Columbian Spirit), shake again and let stand in a hydrometer jar of water at a temperature of about 30° C. (86° F.) for about 10 minutes, or until the clear ether-fat layer has completely separated. Now draw off by means of a nipple pipette, or dropper, an aliquot part of the layer and transfer it to a weighed watch glass or small beaker, washing out the pipette with a little pure ether, dry and weigh. The weight of the watch-glass deducted from this last weight gives the fat in the portion drawn off.

Example.—The ether-fat layer in a sample measured 5.2 c.c. in the burette. 3 c.c. were drawn off and after drying and weighing was found to contain 0.219 grms. of fat. The fat contained in the 5.2 c.c. was found by the following proportion:

$$3 : 5.2 :: .219 : x = 0.380.$$

The sp. gr. of the original milk was 1032, and the weight of milk taken was therefore 10.32 grms. The per cent. of fat was found by the following statement:

$$10.32. : 380 :: 100 \text{ per cent.} : x = 3.68 \text{ per cent.}$$

The volume of the ether-fat layer was found not to be proportional to the fat present, but it invariably contained all the fat. The amount of NaOH added must be adjusted so that the proteids precipitated by the alcohol are in a gelatinous and not lumpy condition.

These short methods will be found useful in the examination of human milk, where long, tedious processes are not likely to be entered into. While some of them are not scientifically accurate, they are sufficiently so for clinical purposes and for the use of sanitary inspectors in sorting milks.

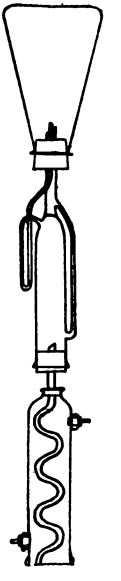


FIG. 46.

The Adams Method.—In this method the milk is absorbed by bibulous paper, previously thoroughly exhausted with ether and alcohol. This paper is usually cut in the shape of long strips, and these are rolled into a coil and put in a special apparatus known as an extractor, and shown in Fig. 8. The coil is put into the chamber of the middle piece of the apparatus, which is then connected with a condenser, as shown. Sufficient ether to fill this chamber is put into the flask below, which is gently warmed. The ether distils up into the condenser and runs back upon the coil, filling the chamber until it flows over through the siphon-tube into the flask below. This is repeated until exhaustion is complete. The ether is finally distilled off, and the fat in the flask is dried and weighed. The results obtained by this method are about 0.3 per cent. higher than those obtained by the method above described, of evaporating in a platinum dish and treating with ether. This has been adopted by official chemists, both in England and in this country, as the standard method of estimating fat. For the accurate estimation of fat in milk in well-equipped laboratories, it leaves little to be desired but is difficult without these facilities.

The Estimation of Sugar. — A sufficiently correct estimation of milk-sugar for clinical purposes can be made by exhausting the residue that remains after the extraction of the fat from the dry solids, with boiling 50 per cent. alcohol. This dissolves the sugar and the soluble portion of the ash. The solution is filtered, evaporated to dryness in a platinum or a porcelain capsule, and weighed. The residue is then ignited and the ash weighed and deducted from the weight of sugar and ash, to obtain the amount of sugar.

Lactose may also be estimated with Fehling's solution, after coagulation and removal of the caseinogen with acetic acid.

For this purpose 15 c.c. of the milk are put into a centrifuge tube and the fat separated as completely as possible. 5 c.c. of the skim-milk are drawn out with a pipette, the cream adhering to the outside of the pipette wiped off, and this milk delivered into a 50 c.c. graduated flask, and diluted with water to about 40 c.c. The contents of the flask are now heated to boiling, a few drops of acetic acid added to precipitate the casein and albumin, cooled and made up to 50 c.c. The mixture is then filtered through a dry filter into a dry flask, or into a burette.

The remainder of the process is conducted as in the estimation of sugar in the urine. (See page 76.) Ten c.c. of Fehling's solution are decolorized by .0676 grms. of milk sugar instead of .050 grms. of glucose. The number of c.c. of the milk serum used will then contain the above amount of sugar.

Example: A certain milk when treated as above, required 12.5 c.c. to decolorize 10 c.c. of Fehling's solution, and therefore the whole 5 c.c. taken contained $4 \times .0676 = .2704$ grms. of sugar.

The specific gravity of the original skim-milk was 1.033 and the 5 c.c. taken weighed $5 \times 1.033 = 5.165$ grms. The per cent. of sugar is found by the following proportion: $5.165 : .2704 :: 100 : x = 5.23$ per cent.

Estimation of the Proteids. — The proteids are often determined by the difference between the total solids and the sum of the other solids. This method is unreliable, because there are present in milk certain extractive matters, sometimes amounting to nearly one per cent. which by this method are calculated as proteids.

While the direct estimation of proteids in milk is of great importance, an easy and reliable clinical method is not at hand.

The nearest approach to such a method is that of Woodward. He employs two short burettes holding 5 c.c. each. These are filled with the milk under examination, and allowed to stand in a warm room, to favor fermentation, until the milk turns sour (18 to 24 hours) and the curd begins to separate. Exactly 5 c.c. of the milk are now drawn into a graduated 15 c.c. centrifuge tube, and 10 c.c. of Esbach's picric acid solution added (see p. 73). The two tubes thus prepared are placed in the centrifugal machine and revolved until a constant reading is obtained, when the percentage is read off. This process has the merit of being easy to conduct, and the author claims that it gives satisfactory results for the clinical examination of human milk.

It does not, however, give us any idea of the composition of the proteids present. This information may be obtained, however, if about 8 c.c. of the milk be taken and the souring be allowed to proceed until the curd separates from the whey very distinctly, and 5 c.c. of the contents of one of the burettes be filtered to remove the curd and fat, before the after treatment with Esbach's reagent. The unfiltered tube will then give the total proteids while the filtered tube will show the lactalbumin and lactoglobulin. When modified in this way the process may give useful information when applied to human milk or modified milk mixtures. The author has found it best to warm the milk to about 40° C. to 50° C. before filtering, in the modified process.

The results are not strictly exact, but will often be found useful.

When the direct determination is desired, they may be precipitated by tannin, filtered, the precipitate dried, and washed with a mixture of one part of alcohol to three of ether until the washings show no trace of tannin. The residue is then dried and weighed.

Ritthausen's Method. — The albuminoids can also be determined by the method of Ritthausen, who employs a solution of $CuSO_4$, containing 6.5 grm. of the crys-

talline salt to the liter, and a solution of alkali of the strength of 14.2 grm. of KOH or 10.2 grm. of NaOH to the liter. The copper salt precipitates the proteids together with the fat. Twenty c.c. of milk are taken, and diluted with water to 400 c.c.; 10 c.c. of the copper solution are then added, with constant stirring, until the coagulum settles and the supernatant liquid is clear. The alkali solution is now added until the liquid is neutral, and the contents of the beaker are filtered, using a previously dried and weighed filter-paper. The precipitate is all transferred to the filter. It is washed first with water, then with diluted alcohol, and finally with ether, until all fat is removed. The remaining precipitate is again washed with alcohol and dried at 110° C. (230° F.) and weighed. The bluish mass is burned, and the loss, after deducting the weight of the filter-paper, is reckoned as caseinogen and albumin.

Estimation of Caseinogen and Albumin. — In the examination of human milk, it is a matter of great importance to know the relative proportion of albumin and caseinogen, because of the great importance of this ratio in infant nutrition. A rapid and easy method of separating these proteids for analytical purposes is very desirable, though none such has been thus far devised. The most of the methods that have been proposed require too much time for clinical purposes. Berggran and Winkler have proposed a volumetric method based upon the fact that the proteids of milk absorb a definite amount of iodine, which seems to give fairly good results.

The following solutions are required:

No. 1. **Iodine Solution.** — Dissolve 12.5 grms. of pure iodine in 250 c.c. of 95 per cent. alcohol. Dissolve 15 grms. of mercuric chloride in 250 c.c. of 95 per cent. alcohol. The latter solution, if necessary, is filtered, and then the two solutions are mixed, and allowed to stand twelve hours before using.

No. 2. **Decinormal Sodium Thiosulphate Solution.** — Dissolve 12.3 grms. of freshly pulverized crystals of pure sodium thiosulphate, after drying well by pressure between blotting paper or filter paper, in water and dilute to exactly 500 c.c.

No. 3. **Potassium Iodide Solution.** — Dissolve 50 grms. of potassium iodide in 500 c.c. of water.

No. 4. **Starch Solution.** — Shake up one grm. of starch in 100 c.c. of water and boil the mixture for ten minutes. Dilute to about 200 c.c. and filter.

No. 5. **Potassium Dichromate Solution.** — This is made by dissolving 1.937 grms. of chemically pure crystallized potassium dichromate in 500 c.c. of distilled water.

This solution is used to standardize solution No. 2. If the thiosulphate solution be freshly prepared with care, this check may be dispensed with. As solutions of $Na_2S_2O_3$ are not very stable, the author prefers to make it fresh when needed. For this purpose the quantity necessary for 100 c.c. of the solution (*i. e.*, 2.460 grms.) is carefully weighed out in a series of small vials well stoppered and kept ready for use in this form.

Estimation of the Total Proteids and Fat in the Same Quantity. — This process requires 10 c.c. of milk, which is measured into a burette. For convenience the burette should have a capacity of 30 c.c. and should be graduated down to the stopcock.

The fat is to be dissolved out of the milk before estimating the proteids. This is accomplished as described on page 168 for the estimation of the fat. After drawing off as much of the fat as can safely be done, and the precipitated proteids have settled down somewhat, the stopcock is opened and the fat-free contents of the burette are run into a four-ounce glass-stoppered bottle into which exactly 10 c.c. of the iodine solution has previously been measured, taking care to run out all the flakes of precipitated proteids, but none of the ether-fat layer.

During this time the exact relative value of 10 c.c. of the iodine solution and the thiosulphate solution is determined by titration of the former with the latter. The titration is made under the same conditions as the analysis, *i. e.*, to 10 c.c. of the iodine solution is added 10 c.c. of ether and 10 c.c. of methyl alcohol, 10 c.c. of the potassium iodide solution, a few drops of starch and water enough to make the whole volume up to 100 c.c. After standing an hour the free iodine, or the excess not taken up by the proteids in the milk, is estimated as follows: To the solution is added 10 c.c. of solution No. 4, and water enough to make the whole measure about 100 c.c. Solution No. 2 is then run in from a burette until the most of the iodine has disappeared and but a faint yellow tint remains. A few drops of the starch solution are now run in and the titration cautiously continued until all blue color is discharged.

Since 1 c.c. of the thiosulphate solution is equivalent to .0127 grms. of iodine, the difference between the number of c.c. of this solution required by 10 c.c. of the iodine solution, and the number required to absorb the excess of iodine in the analysis, multiplied by .0127 gives the amount of iodine absorbed by the proteids of 10 c.c. of the milk under examination. By direct experiments it was determined that the proteids of human milk absorb 33.93 per cent. of their weight of iodine, while the proteids of cow's milk absorb 33.75 per cent.

The proteids can then be calculated from the iodine absorbed, by the following proportion:

33.75, or 33.93 : 100 : : grms. of iodine absorbed : the grms. of proteid present.

Or, 1 grm. of proteid N is equal to 2.61 grms. of iodine.

Estimation of the Albumin. — To 15 c.c. of the milk, warmed to 40° C., add 3 or 4 drops of a 10 per cent. acetic acid, and stir well. Filter off 10 c.c. through a dry filter and neutralize with NaOH solution, transfer to the burette and estimate the proteid in this filtrate exactly as above described. The acetic acid and the NaOH solution should be balanced in strength by a titration. The result thus ob-

tained, deducted from the total proteids obtained by the first titration, gives the caseinogen. The caseinogen of normal human milk is about one third of the total proteid. In cow's milk the caseinogen is usually about five times as much as the albumin.

Detection of Impure Water in Milk. — The addition of water to milk, if it be pure water, can be regarded as harmless to adults. It is rather a sophistication than a harmful adulteration. As this is usually well-water, which may itself be impure, it becomes a matter of importance, because the water may carry with it germs of typhoid fever, cholera, or other diseases, and will impart to the milk infectious properties.

To detect impure water in milk the following process may be used: The milk is coagulated with acetic acid and filtered. To a suitable quantity of the whey add an equal volume of a solution of naphthylamin sulphate and a few c.c. of a freshly prepared solution of sulphanylic acid in sulphuric acid. The test may be made in an ordinary test-tube or in a cylinder. If the milk contains nitrites, due to an impure water, a rose-red color will appear, varying in intensity with the amount of nitrites present, and deepening on standing. The test is very delicate. The following may also be employed: 100 c.c. of the milk are boiled with 1.5 c.c. of a 5 per cent. solution of $CaCl_2$, and filtered. A small portion of the filtrate is treated with H_2SO_4 containing 2 per cent. diphenylamin. This mixture is then floated upon concentrated H_2SO_4, when, if nitrates or nitrites be present in the milk, a blue zone will appear at the line of contact of the two liquids. Or, the test may be applied as follows: 1 c.c. of a solution of diphenylamin in H_2SO_4, is placed in a small porcelain dish, and a few drops of the milk allowed to flow down the side into the acid. If the milk contains nitrites or nitrates, a blue color will appear at the line of separation between the acid and the milk. This test is very delicate, and will detect the presence of a very small quantity of impure water. Nitrites and nitrates are not found in milk, even if contained in the food of the cows.

Determination of the Duration of Lactation. — For this purpose Umikoff suggests the color produced by ammonia in human milk. To 5 c.c. of the human milk to be tested, add 2.5 c.c. of a 10 per cent. ammonium hydroxide solution, and warm the mixture to 60° C. for fifteen to twenty minutes. Human milk when so treated assumes a reddish-violet color, the intensity of which increases with the duration of lactation, from rose-violet to dark-brown violet.

Condensed Milk. — Owing to the difficulty of keeping ordinary milk, several processes of preserving it by concentration have been employed. When milk is simply evaporated, without the addition of

a preservative, it is called **condensed milk.** This is also put into the market sometimes under the name of **evaporated cream.** This term is also applied to what properly should be termed **preserved milk,** or milk which has been condensed, with the addition of cane-sugar. Analyses made by Cornwall, of the condensed milks found in the American market, showed the following average:

Water,	26.95 per cent.	Milk-sugar,	13.38 per cent.
Milk solids,	34.36 "	Cane-sugar,	38.82 "
Casein and albumin,	9.25 "	Ash,	1.92 "
Fat,	9.69 "		

Calculating from these results, he found that the condensation varied from 2.27 to 3.12 times, the average of all analyses being about 2.74 times, or the millk was condensed to not quite one-third the original volume.

Modified Milk. — By this term is meant cows' milk that has been changed in composition so as to resemble the composition of human milk. As the caseinogen of cows' milk is about four times that of human milk, and the albumin is but half that of human milk, there is a very decided difference in the digestibility of the two. Rennin coagulates the caseinogen only. The curd formed in the stomach from cows' milk is more abundant, and forms tough masses, difficult to digest, while that from human milk is slight in amount and flocculent.

The sugar of cows' milk is present in about 4.9 per cent., while that of human milk is nearly 2 per cent. higher. These and other differences in composition and behavior of the two milks, shown in the table on page 157, make it necessary to modify the composition of cows' milk for the successful nourishment of infants. Various methods have been proposed for accomplishing this, and have led to the production of numerous preparations put upon the market as baby-foods.

One of the very generally used methods of modifying cows' milk to imitate human milk, is a mixture of milk, cream, water, lime-water, and milk-sugar, made to correspond to the average gross analysis of human milk.

The milk, as well as the cream, used for this process, must be fresh, and the cream should be of nearly constant composition. The only way to secure this is to use cream separated by the centrifugal machine.

With such a cream, containing 20 per cent. of fat, the following proportions will give nearly the composition of average human milk:

Milk,	2 parts; or,	Milk,	4 fluidounces.
Cream,	3 parts; "	Cream,	6 fluidounces.
Water,	10 parts; "	Water,	20 fluidounces.
Lime-water,	1 part; "	Lime-water,	2 fluidounces.
Milk-sugar,	½ part; "	Milk-sugar,	7 drachms.

The analysis of this mixture will give about the following results, when a good milk is used:

Water,	88.42	Fat,	4	Sugar,	6.26
Solids,	11.58	Proteids,	1.11	Ash,	0.21

A better and more rational method of modifying cows' milk for use as an infant food is the following: The milk should be allowed to stand in a cool place for three or four hours, to allow the cream to separate. When the milk is received in bottles, as is the custom in large cities, this will be unnecessary. Siphon off three fourths of the milk from the bottom of the containing vessel, leaving the cream and upper portion of milk undisturbed. This may be easily done with a small rubber tube, previously filled with water to start the siphonage. Instead of syphoning off the bottom of the liquid, the top may be dipped out with a small dipper known as the Chapin dipper. To the milk thus siphoned off, or to that left in the bottle when the dipper is used, add a teaspoonful and a half of essence of pepsin or liquid rennet, warm to blood heat, 37° C. (98.6° F.), and keep at or near that temperature for ten to fifteen minutes, or until the milk curdles. Then warm, with vigorous stirring, to 68° C. (155° F.) and filter, while hot, through muslin. This whey will contain approximately 0.5 per cent. of fat, 4.9 per cent. of sugar, 0.8 per cent. of albumin, and 0.7 per cent. of salts. When cold, this whey is added to the rich top-milk.

The mixture thus obtained will contain approximately 0.75 per cent. of caseinogen, 0.8 per cent. of albumin, 4.9 per cent. of sugar, 3.7 per cent. of fat, and 0.7 per cent. of salts. To a quart of this mixture we must add 1.5 per cent. of milk-sugar to bring the percentage of this constituent up to 6.4 per cent. This will require about one half ounce, or a heaping tablespoonful, of powdered milk-sugar. This mixture is very successful in practice. The behavior of the mixture, when coagulated with dilute acid, is strikingly like that of human milk. Cereal gruels will sometimes be better tolerated, as the diluent, than either whey or water. These gruels may be made of wheat, barley, rice or oat flour in the proportion of a heaping tablespoonful to the pint of water, and boiled for fifteen to thirty minutes. The gruel may then be dextrinized with diastase, if desired, or, for children over six months of age, they may be used without this.

The upper 8 oz. (upper one-fourth) from a bottle of well-creamed milk, containing 4 per cent. fat, will contain approximately 14 per cent. of fat.

The upper one-third of such a milk will contain about 9.5 to ten per cent. of fat.

The upper half of a bottle of such milk will contain from 7.5 to 8 per cent. of fat.

For home modification these three grades of top-milk will serve all practical purposes.

The following table shows the percentages of the various constituents to be obtained by the mixtures given, in column (*a*) with water, (*b*) with whey, and (*c*) with cereal gruel as the diluents:

COMPOSITION OF MIXTURES OF TOP-MILK WITH WATER (*a*), WHEY (*b*), BARLEY GRUEL (*c*) AND (*d*).

Top-milk 14% Fat.	Diluent.	Fat.			Caseinogen.			Albumin			Barley Proteid.	Lactose.			Malt Sugar.	Salts.	
		a	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*		*a*	*b*	*c*	*d*	*a*	*b*
1	3	3.6	3.7	3.6	0.75	.75	.75	.12	.77	.30	.18	1.22	4.9	1.22	1.52	.15	.70
1	4	2.9	2.9	2.9	.56	.60	.56	.10	.79	.30	.20	.98	4.9	.98	1.6	.12	.70
1	5	2.4	2.4	2.4	.48	.50	.48	.08	.81	.28	.20	.81	4.9	.81	1.8	.10	.70
10 per cent.	milk.																
1	2	3.33	3.43	3.33	1.0	1.0	1.0	.16	.75	.32	.16	1.96	4.9	1.96	1.5	.22	.70
1	3	2.62	2.62	2.62	.75	.75	.75	.12	.77	.30	.18	1.22	4.9	1.22	1.5	.17	.70
1	4	2.0	2.1	2.0	.60	.60	.60	.10	.79	.29	.19	0.98	4.9	0.98	1.6	.14	.70
8 per cent. top	milk.																
1	1	3.9	4.0	3.9	1.5	1.5	1.5	.25	.68	.37	.12	2.45	4.9	2.45	1.1	.25	.70
1	2	2.6	2.6	2.6	1.0	1.0	1.0	.16	.75	.32	.16	1.63	4.9	1.63	1.5	.23	.70
1	3	2.0	2.1	2.0	.75	.75	.75	.12	.77	.30	.18	1.22	4.9	1.22	1.52	.18	.70
Human	milk.	3.7			0.5			1.0				6.5			cane 0.2		
Condensed	milk.														sugar.		
1	4	1.94			1.58			.27				2.48			7.76	.38	

A heaping tablespoonful of lactose per quart of food adds 1.5%.

The white of one egg adds 0.3% of albumin to the quart.

TABLE OF WEIGHTS AND MEASURES.

ENGLISH WEIGHTS.

TROY WEIGHT OR APOTHECARIES' WEIGHT (U. S. P.).

℔ *Pound.*	℥ *Ounces.*	ʒ *Drachms.*	℈ *Scruples.*	gr. *Grains.*		gm. *Grams.*
1	12	96	288	5760	=	373.2419
	1	8	24	480	=	31.1035
		1	3	60	=	3.8879
			1	20	=	1.2959
				1	=	0.0648

AVOIRDUPOIS WEIGHT.

Pound.	*Ounces.*	*Drachms.*	*Grains.*		*Grams.*
1	16	256	7000.	=	453.5926
	1	16	437.5	=	28.3495
		1	27.343	=	1.7718

APOTHECARIES' OR WINE MEASURE (U. S. P.).

C. *Gallon.*	O. *Pints.*	f℥ *Fl. Ozs.*	f ʒ *Fl. Drachms.*	♏ *Minims.*		*c.c.*
1	8	128	1024	61440	=	3785.
	0.1	16	128	7680	=	473.
		1	8	480	=	29.57
			1	60	=	3.70
				1	=	0.06

IMPERIAL MEASURE.

Adopted by the British Pharmacopœia.

Gallon.	*Pints.*	*Fl. Ozs.*	*Fl. Drachms.*	*Minims.*		*c.c.*
1	8	160	1280	76800	=	4543.5
	1	20	160	9600	=	567.9
		1	8	480	=	28.4
			1	60	=	3.55
				1	=	0.06

METRIC MEASURES.

MEASURES OF LENGTH.

1	Millimeter	=	0.001 of a meter.	
1	Centimeter	=	0.010 of a meter.	
1	Decimeter	=	0.100 of a meter	= about 4 inches.
1	**Meter**	=	**1.000 Meter**	= **39.37 inches.**
1	Decameter	=	10.000 meters.	
1	Hectometer	=	100.000 meters.	
1	Kilometer	=	1000.000 meters	= about ⅝ of a mile.
1	Myriameter	=	10,000.000 meters	= about 6½ miles.

MEASURES OF SURFACE.

1	Centaire	=	1 square meter	= about 1⅕ square yards.
1	**Are**	=	**100 square meters.**	
1	Hectare	=	10,000 square meters	= about 2½ acres.

MEASURES OF VOLUME.

1	Cubic centimeter	=	0.001 of a liter.
1	Liter (cubic decimeter)	=	1000 cubic centimeters.
1	Cubic meter	=	1000 cubic decimeters.
1	Cubic meter	=	1000 liters, or 1 kiloliter.
1	Cubic meter	=	1 stere.

MEASURES OF WEIGHT.

1	Milligram	=	0.001 of a gram	— about $\frac{1}{65}$ of a grain.
1	Centigram	=	0.010 of a gram.	
1	Decigram	=	0.100 of a gram.	
1	**Gram**	=	**1.000 Gram**	= **about 15½ grains.**
1	Decagram	=	10.000 grams.	
1	Hectogram	=	100.000 grams.	
1	Kilo(gram)	=	1000.000 grams	= about 2⅕ pounds.
1	Tonneau	=	1000.000 kilos	= about 1 ton.

ALPHABETICAL TABLE OF EQUIVALENT MEASURES.

1 Are . . . . . = 100 sq. meters = 119.6 sq. yards.
1 Barrel (wine) . . . . . = 1.192 hectoliters.
1 Barrel (imperial) . . . . . = 1.635 hectoliters.
1 Bushel (dry) . . . . . = 35.243 liters.
1 Centimeter . . . . . = $\frac{1}{100}$ meter = 0.3937 in.
1 Cubic centimeter . . . . . = 16.2 minims = 0.06102 cu. in.
1 Cubic centimeter of dist. water at 4° C. . . . . weighs 1 gram.
1 Cubic decimeter (1 liter) (1000 c.c.) of dist. water . . . . . weighs 1 kilogram.
1 Cubic decimeter (imperial measure) . . . . . = 61.03 cu. in. = 0.8804 qt.
1 Cubic decimeter (American wine measure) . . = 33.8 fluidounces, or 1.056 qts.
1 Cubic foot . . . . . = 1628 cu. in. = 28,315.31 c.c.
1 Cubic foot of water at 62° F. (16.6° C.) . . . . . weighs 62.32 lbs. av.
1 Cubic inch . . . . . = 266 minims = 16.386 c.c.
1 Cubic inch of water at 62° F. (16.6° C.) . . weighs 252.46 grs. = 16.372 grams.
1 Cubic meter (1 stere) . . . . . = 1000 liters = 35.30 cu. ft.
1 Drachm (troy) . . . . . = 3.888 grams = 60 grains.
1 Fluidrachm . . . . . = 60 minims = 3.697 c.c.
1 Fluidounce (imperial) . . . . . = 28 4 c.c. = 1.7329 cu. in.
1 Fluidounce (wine measure) . . . . . = 29.57 c.c. = 1.8047 cu. in.
1 Fluidounce of water (wine measure) at 62° F. . . . . weighs 456 grains.
1 Fluidounce of water (wine measure) at 60° F. . . . . weighs 29.57 grams.
1 Fluidounce of water (imperial) at 62° F. . . . . weighs 437.5 grains.
1 Foot (12 inches) . . . . . = 34.48 centimeters.
1 Gallon (imperial) . . . . . = 277.27 cu. in. = 4.545 liters.
1 Gallon (wine) . . . . . = 231 cu. in. = 3.785 liters.
1 Gallon of water (imperial) . . . . . weighs 10 lbs.; wine, gallon, 8.34 lbs.
1 Grain (troy) . . . . . = 0.0648 gram.
1 Gram (weight of 1 c.c. of water at 4° C., 39.2° F.) . . . . = 15.4323 grains.
1 Inch . . . . . = 2.54 centimeters.
1 Kilogram . . . . . = 1000 grams = 2.7 lbs. troy = 2.2046 lbs. av.
1 Liter (see cubic decimeter) . . . . . = 61.027 cu. in.
1 Meter (one forty-millionth of earth's meridian) . . . . . = 39.3708 in.
1 Minim . . . . . = 0.0616 c.c. 1 minim of water weighs 0.95 grain.
1 Ounce (troy) . . . . . = 480 grains = 31.1 grams.
1 Ounce (avoirdupois) . . . . . = 437.5 grains = 28.35 grams
1 Pint (imperial) . . . . . = 20 fluidounces = 567.93 c.c.
1 Pint (wine measure) . . . . . = 16 fluidounces = 473.15 c.c.
1 Pound (troy) . . . . . = 5760 grains = 373.24 grams.
1 Pound (avoirdupois) . . . . . = 7000 grains = 453.59 grams.
1 Quart (imperial), 40 fluidounces . . . . . = 69.32 cu. in. = 1.1358 liters.
1 Quart (wine measure), 32 fluidounces . . . . . = 58.30 cu. in. = 0.9463 liter.
1 Ton (avoirdupois) . . . = 2000 lbs. = 29,167 ounces troy = 907.20 kilograms.
1 Tonneau . . . . . = 1,000,000 grams = 1000 kilos = 2204.6 lbs. av.

TABLE OF THE CHEMICAL ELEMENTS WITH THEIR SYMBOLS AND ATOMIC WEIGHTS O = 16.

Aluminum	Al	27.1	Neodymium	Nd	143.6
Antimony	Sb	120.2	Neon	Ne	20
Argon	A	39.9	Nickel	Ni	58.7
Arsenic	As	75.0	Nitrogen	N	14.04
Barium	Ba	137.4	Osmium	Os	191
Bismuth	Bi	208.5	Oxygen	O	16
Boron	B	11	Palladium	Pd	106.5
Bromine	Br	79.96	Phosphorus	P	31
Cadmium	Cd	112.4	Platinum	Pt	194.8
Caesium	Cs	132.9	Potassium	K	39.15
Calcium	Ca	40.1	Praseodymium	Pr	140.5
Carbon	C	12	Radium	Ra	225
Cerium	Ce	140.25	Rhodium	Rh	103
Chlorine	Cl	35.45	Rubidium	Rb	85.4
Chromium	Cr	52.1	Ruthenium	Ru	101.7
Cobalt	Co	59	Samarium	Sm	150
Columbium	Cb	94	Scandium	Sc	44.1
Copper	Cu	63.6	Selenium	Se	79.2
Erbium	Er	166	Silicon	Si	28.4
Fluorine	F	19	Silver	Ag	107.93
Gadolinium	Gd	156	Sodium	Na	23.05
Gallium	Ga	70	Strontium	Sr	87.6
Germanium	Ge	72.5	Sulphur	S	32.06
Glucinium	Gl	9.1	Tantalum	Ta	183
Gold	Au	197.2	Tellurium	Te	127.6
Helium	He	4	Terbium	Tb	160
Hydrogen	H	1.008	Thallium	Tl	204.1
Indium	In	114	Thorium	Th	232.5
Iodine	I	126.85	Thulium	Tm	171
Iridium	Ir	193	Tin	Sn	119
Iron	Fe	55.9	Titanium	Ti	48.1
Krypton	Kr	81.8	Tungsten	W	184
Lanthanum	La	138.9	Uranium	U	238.5
Lead	Pb	206.9	Vanadium	V	51.2
Lithium	Li	7.03	Xenon	Xe	128
Magnesium	Mg	24.36	Ytterbium	Yb	173
Manganese	Mn	55	Yttrium	Yt	89
Mercury	Hg	200	Zinc	Zn	65.4
Molybdenum	Mo	96	Zirconium	Zr	90.6

LIST OF REAGENTS REQUIRED FOR URINE ANALYSIS.

(Numbers indicate page of text.)

Acid, acetic,
" citric,
" hydrochloric, C. P.,
" metaphosphoric, 72
" nitric,
" phosphotungstic,
" picric, see page 71, 89
" salicyl-sulphonic, 72, 74
" sulphuric,
" sulphanilic, 101
" tannic,
" trichloracetic, 72
Alcohol ,
" amylic,
Alizarin, 60
Almen's solution, 75
Alpha-naphthol, 90
Ammonium oxalate, 5%,
" chloride, 10%,
" hydroxide,
" sulphate (cryst.),
Barium acetate, 60
" chloride, 10%,
Bismuth subnitrate,
Bromine, 62
Carbon disulphide, 103
Chloroform, 100
Cochineal, 58
Chlorinated soda solution (squibb) 62, 100
Copper sulphate, 10%,
Ehrlich's solutions, 101
Esbach's solution, 73
Ether,
Fehling's solution, 91
Ferric chloride, 10%,
Haines' solution, 88
Hydrogen dioxide, 78
Indigo carmine, 89
Indigo extract, 89
Iodine,
Iodine, sol. in potass. iodide, 94
Lead acetate, 10%,
" subacetate,
Litmus paper, red and blue,
Magnesium mixture, 66
Magnesium sulphate, (cryst.),
Mercuric chloride. 72
Methylene blue, 90, 102
Nylander's solution, 89
Oliver's solution, 97
Phenolphthalein,
Phloroglucin, 93
Phenol-hydrazin, 90
Potassium bromide, 20%, 63
" chromate,
" hydroxide,
" ferrocyanide,
" sulphocyanate,
Safranin, 90
Sodium acetate,
" carbonate,
" chloride,
" hydroxide,
" nitroprusside, 94
" phosphate,
" potass, tartrate,
" sulphide,
" tungstate, 71
Tauret's solution, 72
Tincture of cochineal, 58
" guaiacum, 78
" iodine, 97
Turpentine, 78
Uranyl nitrate, 58

VOLUMETRIC SOLUTIONS.

Barium acetate (p. 60)	1 c.c. = .0958 grms. $Ba(C_2H_3O_2)_2$.
	1 c.c. = .003 grms. SO_3.
Hydrochloric acid (decinormal)	1 c.c. = .00365 grms. HCl.
Fehling's solution (p. 91)	1 c.c. = .005 grms. diabetic sugar.
	1 c.c. = .00676 grms. lactose.
Iodine solution (p. 67) Ruemann.	
Potass. sulphocyanate (p. 57)	1 c.c. = .00354 grms. chlorine.
	1 c.c. = .00584 grms. NaCl.
Silver nitrate (p. 56) (decinormal)	1 c.c. = .01696 grms. $AgNO_3$.
	1 c.c. = .00584 grms. NaCl.
Silver nitrate (p. 66) ($\frac{1}{30}$ normal)	1 c.c. = .00336 grms. uric acid.
Silver nitrate (p. 68) Hall.	
Sodium hydroxide (decinormal)	1 c.c. = .004 grms. NaOH.
	1 c.c. = .00365 grms. HCl.
Sodium phosphate (p. 58)	1 c.c. = .010085 grms. $Na_2HPO_4.12H_2O$.
	1 c.c. = .002 grms. P_2O_5.
Uranyl nitrate (p. 58)	20 c.c. = 50 c.c. of Na_2HPO_4 solution.
	1 c.c. = .005 grms. P_2O_5.

INDEX.